VOLUME 31 JANUARY, 1953 NUMBER 1

Canadian Journal of Botany

Editor: D. L. BAILEY

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

CANADIAN JOURNAL OF BOTANY

(*Formerly Section C, Canadian Journal of Research*)

The Canadian JOURNAL OF BOTANY is published bimonthly by the National Research Council of Canada under the authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. Matters of general policy are the responsibility of a joint Editorial Board consisting of members of the National Research Council of Canada and the Royal Society of Canada.

The National Research Council of Canada publishes also: *Canadian Journal of Chemistry, Canadian Journal of Medical Sciences, Canadian Journal of Physics, Canadian Journal of Technology, Canadian Journal of Zoology.*

EDITORIAL BOARD

Representing

NATIONAL RESEARCH COUNCIL	ROYAL SOCIETY OF CANADA	
DR. J. H. L. JOHNSTONE (*Chairman*), Professor of Physics, Dalhousie University, Halifax, N.S.	DR. G. M. VOLKOFF, Professor of Physics, University of British Columbia, Vancouver, B.C.	Section III
DR. OTTO MAASS, Macdonald Professor of Physical Chemistry, McGill University, Montreal, P.Q.	DR. T. THORVALDSON, Dean Emeritus of Graduate Studies, University of Saskatchewan, Saskatoon, Sask.	
DR. CHARLES W. ARGUE, Dean of Science, University of New Brunswick, Fredericton, N.B.	DR. D. L. BAILEY, Department of Botany, University of Toronto, Toronto, Ont.	Section V
DR. A. G. MCCALLA, Dean, Faculty of Agriculture, University of Alberta, Edmonton, Alta.	DR. E. HORNE CRAIGIE, Department of Zoology, University of Toronto, Toronto, Ont.	

THE CANADIAN ASSOCIATION OF PHYSICISTS	THE CHEMICAL INSTITUTE OF CANADA
DR. G. M. VOLKOFF, Professor of Physics, University of British Columbia, Vancouver, B.C.	DR. H. G. THODE, Department of Chemistry, McMaster University, Hamilton, Ont.

Ex officio

DR. LÉO MARION (*Editor-in-Chief*), Director, Division of Pure Chemistry, National Research Laboratories, Ottawa.	DR. H. H. SAUNDERSON, Director, Division of Information Services, National Research Council, Ottawa.

Manuscripts should be addressed to:

Dr. Léo Marion,
Editor-in-Chief,
Canadian Journal of Medical Sciences,
National Research Council,
Ottawa, Canada.

Each manuscript should be typewritten, double-spaced, and the original and one extra copy submitted (see **Notes to Contributors** inside of back cover).

Subscriptions, renewals, and orders for back numbers should be addressed to:

Administrative Services,
National Research Council,
Ottawa, Canada.

Subscription rate: $3.00 a year; single numbers: 50 cents. Special rates can be obtained for subscriptions to more than one of the Journals published by the National Research Council.

VOLUME 31 1953

Canadian Journal of Botany

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

CORRECTION

On p. 664 of the September 1952 issue of this journal (Canadian Journal of Botany, Volume 30, Number 5), the sentence just before **Acknowledgment** should read as follows.

Finally, if the pathogen soon disappears from a soil lacking suitable hosts (9 and 10), then the resting sclerotia formed either in the soil or on plant parts late in the season, or the mycelia of virulent races carried to the soil again on planted material, become very important in any disease control measure.

Canadian Journal of Botany

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOLUME 31 JANUARY, 1953 NUMBER 1

THE GROWTH CYCLE OF THE BLUEBERRY AND SOME FACTORS OF THE ENVIRONMENT[1]

BY HUGH P. BELL[2]

Abstract

The effects of latitude, burning, and pruning on the growth cycle of the blueberry were studied and found to be as follows. The morphology of the growth cycle and the sequence of morphological changes were not altered by any of the factors considered. For spring and summer, a north and south difference of one hundred and fifty miles had no greater effect than that which is regarded as "seasonal". Burning and pruning delayed the spring and summer growth by about two weeks. For the late autumn and early winter, there was some evidence that morphological changes occurred in the distal florets during that period, but for the "depth of winter", that is from mid-December till the end of February, the winter bud was a morphological complex that, with the exception of the apical floret, always had the same structure regardless of latitude, burning, or pruning and this structure was the same as that found in normal plants during that season.

Introduction

The normal apical growth of the blueberry, *Vaccinium angustifolium* Ait. var. *laevifolium* House, has been described in a previous article (2). The description there of the death or inactivation of the growing tip twice during each season raises the question:—"Is this growth rhythm fixed and constant or can it be modified by external factors?" The investigation reported below was an attempt to get at least a partial answer to this question.

Material and Procedure

Preliminary Statement

Three external factors have been considered namely, latitude, burning, and pruning.

Latitude

To study the effects of latitude, plants were collected in the following places:—Orangedale 45° 55′ N.; Halifax 44° 38′ N.; and Lockeport 43° 45′ N. The distance due north and south covered by these stations is one hundred and fifty miles. The information gained from these plants was of greatest interest in regard to the winter resting stage, for the times of early vegetative growth, blossoming of the flower, and ripening of the fruit during the spring

[1] *Manuscript received August 25, 1952.*

Contribution from the Department of Biology, Dalhousie University, Halifax, N.S. Supported by grants from the National Research Council of Canada and the Nova Scotia Research Foundation.

[2] *Professor of Botany, Head, Department of Biology, Dalhousie University, Halifax, N.S.*

[The November number of this Journal (Can. J. Botany, 30: 665–789. 1952) was issued November 20, 1952.]

and summer varied from place to place and from season to season. As a rule, this variation was what would be expected, namely, the growth in the south of the province was from one to two weeks in advance of that in the northern part. During certain seasons there were exceptions to this, but the exceptions were obviously due to the weather. Consequently, comparing spring and summer collections from different latitudes did not provide information that was new. However, during the winter, weather conditions were such throughout the province that all the plants were in the dormant or resting stage at the same time. Hence it was for this stage that the plants from the north and south were compared to determine the effect of latitude on the growth cycle. For making this comparison, plants were collected on the same day (Jan. 3, 1951) at all three stations.

Burning

A number of patches were burnt in the vicinity of Halifax. This was done by the recognized method, that is, the straw was spread in the autumn and burning was done in the spring before the frost was out of the ground. The actual dates for burning were as follows:—April 19, 1950; April 16, 1951; April 16 and 19, 1952. For each patch burnt, there was at least one similar patch which could be kept under observation as a control. That the plants of these burnt patches responded to the treatment is indicated by the following gross morphology measurements. One hundred untreated plants and one hundred plants from burnt patches were selected at random. For the normal plants the average length of new wood for the season was 2.55 in. and the average number of flower buds per branch was 1.75. The corresponding figures for the plants from the burnt patches were 7.3 and 4.3. These figures are not meant as an indication of the average response to burning, but merely as a proof that, from the standpoint of gross morphology, there had been a decided response to burning.

Pruning

During the season of 1951, certain patches were pruned at the same time as the other patches were burnt. This pruning was done so as to make the end result as much as possible like burning, that is, the plants were cut to within about two inches of the ground. Later, the length of the new wood for the season was measured and the flower buds counted. The figures obtained indicated that, in gross morphological growth, these plants responded just as the plants of the burnt patches. In regard to density of growth, however, the new shoots were not so numerous as in the burnt patches.

Technique

The methods employed for embedding, etc., were the same as described in the previous article (2).

Findings

Growth During Spring and Summer

It is not necessary to describe the stages or sequence of growth during the spring and summer. It is sufficient to say that, regardless of latitude, burning, or pruning, morphological details and the sequence of growth were always the same and similar to that of the normal plant. This has already been described in detail (2). Regarding the time of development, however, the burnt and pruned patches were at first delayed in their growth. For instance, the appearance of the withered tip was usually about two weeks later in the burnt and pruned patches than in the normal.

Autumn Stage

By October, all the plants had reached the stage usually reached by normal plants. This also was described in the previous article under the heading "Flower Primordia, Later Stage" (2, p. 643). There is no need to repeat this description.

Winter Stage

The winter stage was sufficiently different from the autumn stage to require a detailed description. The method used in its description will be similar to that followed in the previous article. For instance, it will be assumed that the bud contained eight flower primordia and these will be numbered basipetally. Of these primordia, Nos. 1 and 2 varied greatly from bud to bud. No. 1 was nearly always in a very early stage. This varied from a mound of meristematic tissue to a primordium with some of the flower parts evident. Figs. 1 and 2 illustrate typical examples. However, there were a few instances observed where the most distal floret which could be identified was as far advanced as a typical No. 2. This typical No. 2 was usually comparatively small but had all the flower parts differentiated. However, the placentae exhibited no indication of ovule formation and, although the sporogenous tissue of the anther stained deeply, the typical anther wall layers had not been formed. In florets Nos. 3 to 8 all flower parts including the ovules were differentiated, but No. 3 was usually smaller than the others. Fig. 3, which is a drawing of a No. 4 floret, can be regarded as representing the general structure of the florets Nos. 3 to 8. For these florets, the ovaries will be described first and then the stamens. In Nos. 3, 4, and 5 the ovule primordia had the typical initial curvature of an anatropous ovule (Fig. 4) but usually there was no indication of a megaspore mother cell. The basipetally placed florets, Nos. 6, 7, and 8 were slightly more advanced in that certain ovules contained a fully differentiated megaspore mother cell (Figs. 6 and 7). As a rule, there was no indication of integument formation (Fig. 7), but in a few cases in florets Nos. 6 to 8, an integument was differentiated (Fig. 6). For the description of the anthers, the stages reached can be explained most easily by starting with the proximal florets. In these, the wall layers and the

FIG. 1

FIG. 2

FIG. 3

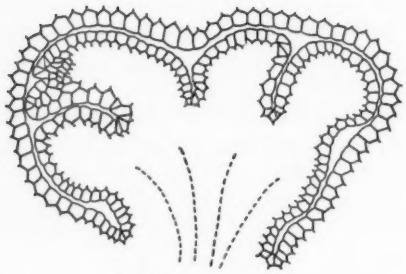


FIG. 4

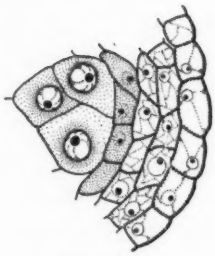


FIG. 5

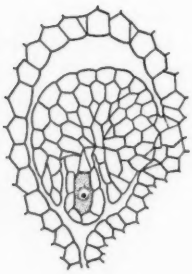


FIG. 6

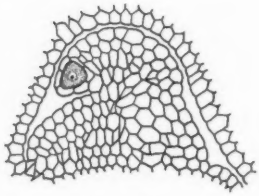


FIG. 7

All figures are camera lucida drawings of the winter resting stage of the blueberry flower bud.

FIGS. 1 and 2. Tangential longitudinal sections of typical apical florets. ×40.

FIG. 1. A very early stage with the first indication of sepal primordia only.

FIG. 2. Both sepal and petal primordia present.

FIG. 3. Median longitudinal section of a No. 4 floret. Sporogenous tissue of the anther cross-hatched. ×40.

FIG. 4. Transverse section of one loculus of an ovary. The ovules on the margin of the placenta have started the curvature typical of an anatropous ovule. ×65.

FIG. 5. Transverse section of a segment of anther wall and three cells of the sporogenous tissue. ×660.

FIGS. 6 and 7. Longitudinal sections of ovule primordia, each with a megaspore mother cell. ×295.

FIG. 6. An integument has been formed.

FIG. 7. No indication of an integument.

tapetum were formed and the sporogenous tissue appeared to be in the microspore mother cell stage, but there was no indication of approaching meiosis (Fig. 5). To check this last point, plants were examined which had been collected during the commencement of growth in the spring, and meiosis was observed to start without any further vegetative cell division. In florets Nos. 4 and 5, the wall layers of the anther did not appear to be as completely differentiated as in the most basipetally placed florets, but the difference was one of cell wall structure only and was so slight that it was difficult to detect with certainty. The description given above is of the buds collected during late December, January, and February and was that found in all buds collected during that period from both normal and treated plants and regardless of latitude, burning, or pruning. This dormant or minimum activity stage appears to be fixed for the species and is independent of external factors. Evidence has been obtained which suggests a similar constancy for the winter buds of other Ericaceae, although the stage reached is different for each species. This point is under investigation.

Growth During the Winter

A slight development during at least the first part of the so-called "dormant season" was suggested by the evidence available. For instance, in most of the October buds examined, floret primordia Nos. 1 and 2 and sometimes No. 3 were in a very early stage of development. In the January and February buds, the usual condition was for only floret No. 1 to be in this very early stage and there were some buds examined which had no undeveloped apical floret. It was not possible to examine a sufficiently large number of buds to make this evidence statistically significant, but there was sufficient evidence to suggest that development of the apical florets may have continued during October, November, and early December. This coincides with Bell's observations on the winter development in the apple bud (1). There, the growth curve continued to rise until the middle of December although it was flat or inconclusive from the middle of December to the end of February. For the blueberry, no change in size or stage of development and no mitotic figures were observed during this latter period which might be called the "depth of winter". These facts, along with the similarity of all mid-winter buds, suggest that the winter condition described above for this last part of the winter, is the real resting stage or stage of minimum activity. However, it may not be in existence for the whole of the season which is regarded as winter, but, from October to December, measurable growth in size may proceed very slowly.

Conclusions

This investigation indicates that although burning and pruning delay the growth in the spring and summer and differences in latitude or weather may advance or retard it, the morphology and sequence of the growth phenomena are constant features, and by the autumn all plants from both south and north

and both normal and treated are at about the same stage. From then on during late autumn and early winter there may be a slow morphological development within the bud. Evidence suggesting this was found in all material collected, but during the last part of the winter, that is, from late December to the end of February, in spite of the differences between florets within each bud, florets Nos. 2 or 3 to 8 are similar from bud to bud and the variation from floret to floret within each bud is constant. That is, with the exception of the one or sometimes two apical florets, the winter bud is a morphological complex which is fixed for the species. That is, in the growth cycle of the blueberry, the morphology of growth changes and their sequence are not changed by any of the factors considered.

Acknowledgments

This work was carried out with the technical assistance of Miss Ann M. Marshall and Miss Jane Burchill. Miss Jane Burchill drew all the figures.

References

1. BELL, H. P. Winter growth in the vegetative buds of the Wagner apple. Can. J. Research, C, 18 : 585-590. 1940.
2. BELL, H. P. Determinate growth in the blueberry. Can. J. Research, C, 28 : 637-644. 1950.

AN ESSENTIAL BACTERIAL GROWTH FACTOR PRODUCED BY MICROBIAL SYNTHESIS[1]

By A. G. Lochhead[2] and Margaret O. Burton[3]

Abstract

An organism isolated from soil, cultivated in a simple medium containing only inorganic salts and sugar, was found to synthesize an essential growth factor that was nutritionally equivalent to soil extract in promoting the growth of an organism for which the latter was hitherto considered essential. The factor could not be replaced by the B vitamins, purines, pyrimidines, or other growth-promoting substances tested (including derivatives of various natural products) with the exception of hemin, which evoked but a partial response, and concentrated liver extract, the effect of which was not attributable to vitamin B_{12}. Partially purified material from the metabolic liquid showed distinct growth-promoting activity at a concentration of 0.1 p.p.m. The organism requiring the growth factor, as well as that synthesizing it, both pleomorphic bacteria of the "soil diphtheroid" type, were found to be hitherto undescribed species. They are considered to be most appropriately assigned to the genus *Arthrobacter*, as proposed by Conn and Dimmick, and are named, respectively, *Arthrobacter terregens* n. sp. and *Arthrobacter pascens* n. sp.

Introduction

Previous studies in this laboratory (15, 16) have indicated the presence in soil of a group of bacteria dependent for growth upon some essential factor or factors contained in soil extract and not furnished by yeast extract or by certain combinations of amino acids or growth factors recognized at the time. It was also shown that for certain strains of bacteria requiring soil extract, the growth-promoting effect of the latter could be replaced by filtrates from cultures of a number of bacteria with simple nutritional needs. For other organisms requiring soil extract similar filtrates were ineffective, pointing to the presence of more than one essential factor.

More recently it was shown (17, 18) that, for an important proportion of the indigenous bacteria of soil dependent upon a factor or factors in soil extract, the growth-promoting effect of the latter could be replaced by vitamin B_{12} and that many other soil bacteria were able to synthesize a factor the nutrilite effect of which was related to the amount of vitamin B_{12} produced. Other more exacting soil bacteria, however, remained dependent upon unidentified growth-promoting substances in soil extract. The present paper, which follows a preliminary report (14), deals with a growth factor found to be synthesized by an organism isolated from soil, which at very low concentrations has been found to be nutritionally equivalent to soil extract in promoting growth of certain organisms for which other growth factors tested have not been found adequate.

[1] *Manuscript received September 4, 1952.*

Contribution No. 346 from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa.

[2] *Chief, Division of Bacteriology and Dairy Research.*

[3] *Assistant Bacteriologist.*

Experimental

Bacteria were isolated from a field soil and differentiated according to their nutritional requirements by methods previously described (18). Special attention was given to organisms requiring soil extract for growth but for which vitamin B_{12} was unable to replace soil extract. In a study of the essential factor in soil extract required by certain of these forms, it was found that culture filtrates of a number of soil bacteria having simple nutritional requirements, in that they were able to grow well in a medium containing only inorganic salts and sugar, were able to promote growth equivalent to that provided by soil extract. More detailed study was therefore made, using a selected test organism requiring the growth factor (No. 88) and one capable of synthesizing it (No. *B*89), both apparently undescribed species.

Recognition of the Growth Factor

In the earlier studies for recognition of the growth response of the test organism (No. 88), a basal medium containing inorganic salts, glucose, and yeast extract (Medium *Y*) was employed (16). Following a determination of the more specific growth factor requirements of the organism (4), a better defined basal medium was developed for assay purposes and used in later work. This consisted of inorganic salts, glucose, casamino acids, and vitamins required in addition to the factor herein described, namely biotin, thiamine, and pantothenic acid. For the assay the ingredients of the basal medium were added to distilled water at 5/4 of the final concentration and a series of 40-ml. quantities measured out, depending upon the number of portions to be tested. After addition of the test materials, the volumes were made up to 50 ml. in all cases and 10-ml. portions added to quadruplicate 50-ml. Erlenmeyer flasks. After sterilization, the contents of the flasks were inoculated with one drop of a suspension of the test organism (No. 88) prepared by centrifuging 10 ml. of a four to five day culture in medium *Y* plus 0.1 ml./100 ml. of culture filtrate of No. *B*89, washing three times in sterile saline. and finally making up to 10 ml. At first cultures were incubated at 26° C, but in later tests at 20° C. After 70 hr. incubation, turbidity measurements were made by a Unicam spectrophotometer at a wave length of 450 mμ, allowing for uninoculated controls. Results are expressed as the average optical density of quadruplicate cultures.

Effect of Soil Extract and Culture Filtrate of Organism B89

Though the growth factor was first recognized by the growth-promoting effect of soil extract* added to the basal medium, it was noted that an active principle, nutritionally equivalent to soil extract, was much more concentrated in culture filtrates of the organism *B*89, grown in a simple medium consisting of potassium monohydrogen phosphate, 1.0 gm.; potassium nitrate, 0.5 gm.; $MgSO_4 . 7H_2O$, 0.2 gm.; calcium chloride, 0.1 gm.; sodium

* *Prepared by autoclaving 1 kgm. field soil with 1 liter tap water for 30 min. at 15 lb., filtering, and making filtrate up to 1 liter.*

chloride, 0.1 gm.; $FeCl_3 . 6H_2O$, 0.01 gm.; glucose, 1.0 gm.; distilled water, 1 liter. This is shown in Table I giving typical results from the addition, respectively, of soil extract and culture filtrate from which the cells had been removed by centrifugation. It is noted that observable growth response is given by additions of the culture fluid at dilutions as great as 1-50,000. The sterilized culture filtrate was found to retain its activity when stored at 4° C. and was used in further tests as a positive control sample.

TABLE I

EFFECT OF SOIL EXTRACT AND CULTURE FILTRATE OF ORGANISM *B89* ON GROWTH OF TEST ORGANISM NO. 88

Addenda to basal medium[1]	Amount per ml.	Optical density (aver. 4 tests)
None	—	.005
Soil extract	0.05 ml.	.099
" "	0.1 "	.130
" "	0.2 "	.146
" "	0.3 "	.171
" "	0.5 "	.114
Culture filtrate, No. *B89*	0.00001 ml.	.007
" " "	0.00002 "	.014
" " "	0.00003 "	.044
" " "	0.00004 "	.083
" " "	0.00005 "	.105
" " "	0.00007 "	.149
" " "	0.0001 "	.180
" " "	0.001 "	.196
Culture medium used for *B89*	0.01 ml.	.004

[1] *Inorganic salts, glucose, yeast extract.*

Effect of Vitamins and other Growth-promoting Substances

The effect of various combinations of vitamins, purines, and pyrimidines on the growth of the test organism is shown in Table II. The vitamins tested, in amounts per 100 ml., were thiamine, 40μgm.; riboflavin, 40μgm.; niacin, 40μgm.; pyridoxine, 40μgm.; pyridoxal, 40μgm.; pyridoxamine, 4μgm.; calcium pantothenate, 40μgm.; biotin, 0.1μgm.; folic acid, 4μgm.; vitamin B_{12}, 0.4μgm.; *p*-aminobenzoic acid, 40μgm.; inositol, 2 mgm.; and choline, 80μgm.; while the purines and pyrimidines consisted of adenine, guanine, xanthine, and uracil, all at a concentration of 1 mgm. per 100 ml. In no case were the combinations used able to promote growth of the test organism, which grew readily upon addition of the culture filtrate of *B89*.

Table II also illustrates the effect of other substances which have been variously reported to exert growth-promoting action upon different bacterial species. However, the substances used showed no effect on the test organism which responded only to additions of the culture filtrate.

TABLE II

EFFECT OF VITAMINS AND OTHER FACTORS ON GROWTH OF ORGANISM No. 88

Addenda to basal medium[1]	Concentration	Optical density (aver. 4 tests)
None	—	.015
Culture filtrate *B89*	0.1 ml./100 ml.	.186
13 Vitamins	See text	.014
13 Vitamins + culture filtrate *B89*		.196
Purines + pyrimidines	See text	.014
Purines + pyrimidines + cult. filt. *B89*		.207
Vitamins + purines + pyrimidines		.009
Vitamins + purines + pyrimidines + culture filtrate *B89*		.213
None	—	.005
Culture filtrate *B89*	0.1 ml./100 ml.	.170
Cytosine	2 mgm./100 ml.	.005
Thymine	2 mgm./100 ml.	.004
Cytosine + thymine + cult. filt. *B89*		.169
Ribonucleic acid	20 mgm./100 ml.	.004
Desoxyribonucleic acid	20 mgm./100 ml.	.005
Ribonucl. a. + desoxyribonucl. a. + culture filtrate *B89*		.169
β-Alanine	10 mgm./100 ml.	.005
Cystine	100 μgm./100 ml.	.004
None	—	.001
Culture filtrate *B89*	0.1 ml./100 ml.	.165
Oleic acid	0.001 ml./100 ml.	.001
Oleic acid + culture filtrate *B89*		.154

[1] *Inorganic salts, glucose, yeast extract.*

A number of less well defined substances, prepared from various natural products, have been found to exert growth-promoting effects on various microorganisms not attributable to known vitamins and related factors of known composition. Thus enzymatic casein digest has been reported by Wright and Skeggs (28) to contain a factor essential for certain strains of streptococci and considered identical with the strepogenin factor of Sprince and Woolley (25) required by various hemolytic streptococci and *Lactobacillus casei*. A factor in clarified tomato juice was found by Shorb (23) to be essential for growth of *Lactobacillus lactis* Dorner, the "TJ" activity being subsequently shown by Hendlin *et al.* (8) to be due to the presence of pyridoxamine phosphate and pyridoxal phosphate. Yeast extract has been reported to contain various unidentified growth factors, including the "citrovorum factor", also present in liver extract, required by *Leuconostoc citrovorum* (21), the "*Lactobacillus bulgaricus* factor" (27), and a factor for *Butyrobacterium rettgeri* (10). The effect of various natural substances or their derivatives is shown in Table III in which it is noted that the materials used failed to promote growth of the test organism.

TABLE III

EFFECT OF DERIVATIVES OF VARIOUS NATURAL PRODUCTS ON GROWTH OF ORGANISM No. 88

Addenda to basal medium[1]	Concentration	Optical density (aver. 4 tests)
None	—	.005
Culture filtrate *B89*	1 ml./100 ml.	.170
Enzymatic casein digest	0.1 gm./100 ml.	.004
Enzymatic casein digest + cult. filt. *B89*		.171
None	—	.015
Culture filtrate *B89*	0.1 ml./100 ml.	.186
Casamino acids	0.1 gm./100 ml.	.015
Casamino acids + cult. filt. *B89*		.214
None	—	.007
Culture filtrate *B89*	10%	.210
Tomato juice, filtered	10%	.006
Tomato juice, charcoal treated, filtered	10%	.004
None	—	.006
Culture filtrate *B89*	0.1 ml./100 ml.	.211
Yeast extract (Difco)	.5%	.007
Peptone	.1%	.009
Peptone	.5%	.010
Tryptose	.1%	.007
Tryptose	.5%	.018
Soil extract ash	Equiv. to 25%	.005

[1] *Inorganic salts, glucose, yeast extract.*

TABLE IV

EFFECT OF HEMIN OF GROWTH OF ORGANISM No. 88

Addenda to basal medium	Optical density (aver. 4 tests)			
Basal medium	*Med. Y*[1]	*Med. CV*[2]	*Med. CV*	*Med. CV*
Date of test	*9.2.52*	*5.8.52*	*10.8.52*	*19.8.52*
None	.004	.003	.006	.010
Culture filtrate *B89* (0.1 ml./100 ml.)	.158	.131	.228	.175
Hemin (1 μgm./100 ml.)	.009	.009	.013	.010
Hemin (10 μgm./100 ml.)	.046	.040	.050	.056
Hemin (50 μgm./100 ml.)	.025	.011	.027	.049
Hemin (100 μgm./100 ml.)	.007	.004	.005	.000
Hemin (10 μgm./100 ml.) + cult. filt. *B89*	—	—	.210	.170

[1] *Inorganic salts, glucose, yeast extract.*

[2] *Inorganic salts, glucose, casamino acids, biotin, thiamine, pantothenic acid.*

For certain species of the genus *Hemophilus* the iron porphyrin, hemin, is known to be an essential growth factor. In tests with organism No. 88 it was found that a partial response resulted from the addition of small amounts of hemin, much less than that provided by the culture filtrate *B*89. Results of four experiments (which included tests with two basal media) are shown in Table IV. The growth-promoting effect of hemin was found to reach a maximum at concentrations of approximately 10-50μgm. per 100 ml., increased amounts of hemin resulting in diminished growth of the test organism in all cases. It is of interest that organism No. 88, a soil form, showed some response to a factor hitherto considered a proved requirement for bacteria only in the case of certain hemophilic species (24). The specificity of hemin as a growth factor for No. 88, however, must await determination of the chemical nature of the factors present in soil extract and in the culture filtrate *B*89.

Effect of Liver Extract and Related Factors

A growth factor effect equivalent to that given by the culture filtrate of organism No. *B*89 was obtained on addition of small amounts of injectable

TABLE V

EFFECT OF LIVER EXTRACT, VITAMIN B_{12} AND FOLIC ACID ON GROWTH OF ORGANISM NO. 88

Addenda to basal medium	Optical density (aver. 4 tests)
None[1]	.005
Culture filtrate *B*89 (0.1 ml./100 ml.)	.196
Liver extract (0.37 mμgm. B_{12}/ml.)	.007
Liver extract (3.75 mμgm. B_{12}/ml.)	.075
Liver extract (37.5 mμgm. B_{12}/ml.)	.228
Liver extract (75.0 mμgm. B_{12}/ml.)	.194
Vitamin B_{12}, crystalline (0.37 mμgm./ml.)	.003
Vitamin B_{12}, " (3.75 mμgm./ml.)	.003
Vitamin B_{12}, " (37.5 mμgm./ml.)	.004
Vitamin B_{12}, " (75.0 mμgm./ml.)	.003
None[2]	.008
Culture filtrate *B*89 (0.1 ml./100 ml.)	.310
Folic acid (50 μgm./100 ml.)	.009
Folic acid (500 μgm./100 ml.)	.005
Folic acid (50 μgm./100 ml.) + cult. filt. *B*89	.242
Folic acid (500 μgm./100 ml.) + cult. filt. *B*89	.240
Folic acid (50 μgm./100 ml.) + purines[3] + uracil[4]	.004
Folic acid (500 μgm./100 ml.) + purines + uracil	.002
Folic acid (50 μgm./100 ml.) + purines + uracil + cult. filt. *B*89	.228
Folic acid (500 μgm./100 ml.) + purines + uracil + cult. filt. *B*89	.238

[1] *Basal medium—inorganic salts, glucose, yeast extract.*
[2] *Basal medium—inorganic salts, glucose, casamino acids, biotin, thiamine, pantothenic acid.*
[3] *Adenine, guanine, xanthine, all 1 mgm./100 ml.*
[4] *1 mgm./100 ml.*

"15 unit" liver extract. The effect, however, is not attributable to vitamin B_{12}, as indicated in Table V, which shows results with different amounts of liver extract and equivalent concentrations of crystalline vitamin B_{12}. Growth stimulation of *Lactobacillus leichmannii* by an unidentified factor in liver extract which promotes growth in excess of that attributable to vitamin B_{12} has been reported by Peeler and Norris (19); however, since this factor was found in tomato juice, as well as in yeast, there is reason for assuming that the factor synthesized by organism *B*89 is a different substance. Concentrates from liver have been found by Sauberlich and Baumann (21, 22) to possess a factor required for the nutrition of *Leuconostoc citrovorum*, also not replaceable by vitamin B_{12} though partially replaceable by thymidine or by high levels of folic acid. Sauberlich (20) furthermore indicated that the factor was involved in purine metabolism by the organism, which required for growth either xanthine, guanine, or hypoxanthine. It was also reported that high amounts of folic acid could, in the presence of purines, replace the unknown factor required by *L. citrovorum*, while further evidence has accumulated supporting the belief that a close relationship exists between the factor and folic acid, which, with the simultaneous addition of thymidine, permitted good

TABLE VI

EFFECT OF ANIMAL PROTEIN FACTOR SUPPLEMENTS AND ANTIBIOTICS ON GROWTH OF ORGANISM NO. 88

Addenda to basal medium	Optical density (aver. 4 tests)
None[1]	.005
Culture filtrate *B*89 (0.1 ml./100 ml.)	.167
Pfizer – Antibiotic feed supplement 'Bi-Con TM-5' (1 mgm./100 ml.)	.005
Pfizer – Antibiotic feed supplement + cult. filt. *B*89	.172
Pfizer – APF supplement 'Bi-Con APF-3' (1 mgm./100 ml.)	.004
Pfizer – APF supplement + cult. filt. *B*89	.222
Lederle – APF supplement (1 mgm./100 ml.)	.005
Lederle – APF supplement + cult. filt. *B*89	.210
Merck – APF supplement (1 mgm./100 ml.)	.004
Merck – APF supplement + cult. filt. *B*89	.178
None[2]	.004
Culture filtrate *B*89 (1 ml./100 ml.)	.156
Aureomycin (0.1 gm./100 ml.)	.004
Aureomycin (1.0 gm./100 ml.)	.003
Aureomycin (0.1 gm./100 ml.) + cult. filt. *B*89	.167
Aureomycin (1.0 gm./100 ml.) + cult. filt. *B*89	.179
Penicillin (0.1 gm./100 ml.)	.005
Penicillin (1.0 gm./100 ml.)	.006
Penicillin (0.1 gm./100 ml.) + cult. filt. *B*89	.163
Penicillin (1.0 gm./100 ml.) + cult. filt. *B*89	.147

[1] *Basal medium—inorganic salts, glucose, casamino acids, biotin, thiamine, pantothenic acid.*
[2] *Basal medium—inorganic salts, glucose, yeast extract.*

growth of the organism (3). Of further interest is the establishment by Bardos *et al.* (1) of the relationship of the citrovorum factor with the folinic acids.

The effect of different concentrations of folic acid, with and without the addition of the purines adenine, guanine, and xanthine (and also the pyrimidine uracil) is shown in Table V. No growth response of the test organism is noted without the inclusion of the culture filtrate *B*89.

The relationship of the growth factor in the culture filtrate to one present in liver extract suggested tests on the effect of various commercial animal protein supplements prepared as by-products of antibiotic fermentation processes and of varying vitamin and antibiotic content. In Table VI are shown results of tests on four commercial preparations which contained, respectively, small amounts of terramycin, streptomycin, aureomycin, and streptomycin. Included in the table are data on the effect of aureomycin and penicillin in pure form, with and without addition of the active culture filtrate *B*89. It is noted that only in the presence of the latter was there any growth response of the test organism.

Growth Factor Synthesized by Organism *B*89

Organism No. *B*89 was isolated by plating on soil extract agar from the same sample of soil from which No. 88 was obtained, but, in contrast to the latter, was found to have simple nutritional requirements, showing good growth in a medium consisting of inorganic salts and glucose with potassium nitrate as sole source of nitrogen. The medium (Med. *B*) was used originally for production of the growth factor and also served, with the addition of agar, as a suitable solid substrate for maintaining stock cultures. It was prepared by adding the following to 1 liter distilled water: potassium monohydrogen phosphate, 1.0 gm.; potassium nitrate, 0.5 gm.; $MgSO_4 . 7H_2O$, 0.2 gm.; calcium chloride, 0.1 gm.; sodium chloride, 0.1 gm., and $FeCl_3 . 6H_2O$, 0.01 gm. The solution was adjusted to pH 6.8, heated to boiling, allowed to cool, and filtered after which 1.0 gm. glucose was added.

Production of Growth Factor

For production of the growth factor, 300-ml. quantities of the medium contained in 1000-ml. Erlenmeyer flasks are inoculated with culture No. *B*89. The flasks are agitated in a reciprocal shaking machine at room temperature, shaken cultures having been found to develop greater activity than stationary cultures, in which latter the growth tends to become somewhat viscous. Though the data in Tables VII and VIII refer to cultures incubated for six days, it was later found that shorter periods were equally satisfactory. At the end of the incubation period the cultures, white to cream colored, are clarified by centrifugation, after which the growth factor potency may be assayed by using culture No. 88.

It was found that the activity of the culture filtrate could be increased somewhat by raising the concentration of potassium nitrate to 0.1% and that of glucose to 0.5%. In a comparison of the effect of different carbohydrates it was noted, as shown in Table VII, that sucrose was somewhat more effective than glucose, and mannitol rather less effective. When lactose or glycerol was used the titer of the active factor was very low though the latter, in contrast to lactose, permitted good growth of the organism.

TABLE VII

EFFECT OF NITROGEN SOURCE AND CARBOHYDRATE ON PRODUCTION OF GROWTH FACTOR BY ORGANISM No. *B*89

Addenda to basal medium ($-KNO_3$)		pH	Turbidity (optical density)	Activity of culture filtrate[1] (optical density)
Nitrogen source	Carbohydrate			
0.1% KNO_3	0.5% Glucose	7.15	.579	.099
0.1% KNO_3	0.5% Sucrose	8.15	.642	.160
0.1% KNO_3	0.5% Lactose	6.6	.018	.003
0.1% KNO_3	0.5% Glycerol	5.1	.490	.017
0.1% KNO_3	0.5% Mannitol	7.4	.499	.080
				.002 (control)
0.05% KNO_3	0.2% Glucose	8.15	.361	.083
0.2 % KNO_3	0.2% Glucose	8.1	.405	.054
0.05% KNO_3 + 0.2% Casamino acids	0.2% Glucose	8.0	.435	.005
0.05% KNO_3 + 0.2% yeast extract	0.2% Glucose	8.2	.595	.002
				.002 (control)
0.1% KNO_3	0.5% Sucrose	8.35	.660	.185
0.1% NH_4Cl	0.5% Sucrose	4.0	.350	.013
0.1% Ammonium citrate	0.5% Sucrose	7.95	.781	.013
0.1% Asparagine	0.5% Sucrose	7.1	.449	.121
0.1% Casamino acids	0.5% Sucrose	6.8	.845	.017
				.010 (control)

[1] *Assay using culture No. 88; 0.01 ml. culture fluid per 100 ml. assay medium; average of four tests.*

TABLE VIII

EFFECT OF HEAVY METALS ON PRODUCTION OF GROWTH FACTOR BY ORGANISM No. *B*89

Addenda to basal medium ($-KNO_3$)			pH	Turbidity (optical density)	Activity of culture filtrate[1] (optical density)
Nitrogen source	Carbohydrate	Metallic salt			
0.1% KNO_3	0.5% Sucrose	None	8.5	.720	.151
"	"	.001% $MnSO_4$	8.3	.662	.052
"	"	.001% $ZnSO_4$	8.45	.570	.136
"	"	.001% $CoCl_2$	7.6	.770	.004
"	"	.001% $CuCl_2$	6.7	.006	.004
					.009 (control)

[1] *Assay using culture No. 88; 0.01 ml. culture fluid per 100 ml. assay medium; average of four tests.*

The influence of different sources of nitrogen on production of the growth factor is also indicated in Table VII. The highest values were obtained by the use of potassium nitrate, whereas ammonium nitrogen, as either ammonium chloride or ammonium citrate, was quite unsuitable. Moderate amounts of the factor were produced with asparagine as source of nitrogen; however, casamino acids, though promoting excellent growth, prevented synthesis of the growth factor. The addition of yeast extract has a similar effect, indicating that more complex media are less suitable than very simple substrates. The data show plainly that the density of growth of organism *B*89 shows no relationship to production of the growth-promoting substance.

The effect of adding small amounts of salts of heavy metals on the elaboration of the growth factor is indicated in Table VIII, which gives the results of tests with salts of manganese, zinc, cobalt, and copper added to the medium in concentration of 0.001%. In all cases the added salts depressed growth factor production, though less noticeably so in the case of zinc. Although excellent growth of the organism occurred in the presence of cobalt, growth factor production was nil; on the other hand, growth was suppressed in the presence of the copper salt.

Concentration of Growth Factor

For concentration of the growth factor the culture fluid is preferably adjusted to pH 5.0-5.5 and the cells removed by centrifugation. The active substance may be removed by treatment with charcoal (Norit) on which it is adsorbed more completely from acid than from alkaline solutions. Elution may be effected by methanol, ethanol, or butanol, preferably somewhat alkaline. Though ethanol elutes rather more completely than butanol, the butanol eluate is usually more potent than those of the other alcohols. Concentration of the eluate gives a brownish colored residue which may show distinct growth-promoting activity at a concentration of 0.1 μgm. per ml.

The active substance is soluble in water and alcohol, sparingly soluble in ether, benzene, and acetone and somewhat more in chloroform, but insoluble in carbon tetrachloride, ethyl acetate, and petroleum ether. Solutions adjusted to pH 2.0 and 10.0 respectively show little loss of activity after standing for 30 hr. Direct extraction of the culture fluid with butanol results in only partial removal of the growth factor; however, the butanol extract shows a high degree of activity. Purification of the active factor has not yet been accomplished.

Observations

The more recent studies on the nutrition of soil bacteria have emphasized the wide diversity in growth requirements shown by various groups of the autochthonous microflora, many of which are dependent upon vitamins and related growth factors known to be present in soil. The occurrence of most of the vitamins of the B group may be attributed to their synthesis by micro-organisms as well as to the presence of vitamin-rich organic matter. Having in mind the widely diverse nutritional needs of even closely related types,

which in many cases can be met only by furnishing soil extract, it is evident that soil contains various unknown growth-promoting substances. It is reasonable to suppose that as the growth-promoting effect of soil extract is resolved into its component factors, it will be possible eventually to develop a defined medium adequate for growth of the most exacting types. The factor synthesized by organism *B*89 is believed to represent a nutrilite contributing to the growth-promoting effect of soil extract. It is considered a matter of further interest that for the test organism it may not only replace soil extract, but is nutritionally equivalent to a factor present in liver extract, and partially replaceable by hemin.

Description of Organisms

Organism No. 88 – Arthrobacter terregens n. sp.

Studies of the morphological and physiological characteristics of the organism requiring the growth factor may be made by employing appropriate solid or liquid media in which soil extract or the culture filtrate of organism No. *B*89 is incorporated. Stock cultures may be maintained on soil extract agar (1.5% agar) or soil extract semisolid (0.3% agar) containing 25% by volume of soil extract and the following: 0.1% potassium monohydrogen phosphate, 0.05% potassium nitrate, 0.02% $MgSO_4 . 7H_2O$, 0.01% calcium chloride, 0.01% sodium chloride, 0.001% $FeCl_3 . 6H_2O$, glucose 0.1%, and yeast extract 0.1%. The soil extract may be replaced by 0.1% of the culture filtrate of *B*89 (*YX* agar), while most of the standard differential media may be made to promote growth, either by substitution of 25% soil extract for water, or by incorporation of 0.1% of culture filtrate of *B*89 and, if necessary, 0.1% yeast extract to furnish other essential growth factors.

Colonies on soil extract agar or *YX* agar are round, entire, convex with glistening surface, yellowish-brown in color, and reach a diameter of 1 to 2.5 mm. Surface streak cultures show moderate, filiform growth, flat, with a mottled 'broken glass' appearance, becoming yellowish-brown in color. There is no growth on nutrient agar, but, with the addition of 0.1% culture filtrate *B*89, growth is similar to that indicated above, though with little chromogenesis. Stab cultures in soil extract semisolid are pale brownish in color, with growth best at the top and spreading over the surface. In nutrient broth no growth is observed, but, on addition of culture filtrate and yeast extract, the organism produces moderate turbidity, without surface pellicle, but with a yellowish, stringy sediment.

Stab cultures in nutrient gelatin prepared with soil extract, or with the addition of culture filtrate *B*89 and yeast extract, show yellowish growth along the line of inoculation, and slightly spreading at the surface. However, no liquefaction occurs on prolonged incubation. Reduction of nitrate to nitrite occurs in nitrate broth with added growth factor. Starch is not hydrolyzed and no change occurs in milk containing the growth factor after two months' observation. Hydrogen sulphide is not produced in lead acetate medium, nor is indole formed under conditions suitable for growth. Though growth

is somewhat improved in the presence of glucose, sucrose, and mannose, and to a lesser degree with lactose, but not with glycerol, only very slight increases in acidity are observed in the presence of the carbohydrates, with no evidence of gas production. Cultures in a suitable medium give negative tests for the methyl red and Voges-Proskauer reactions. The temperature range for growth extends from 15° to 35° C. with no growth occurring at 10° or at 37° C. Growth is best at 20° to 25° C., being rather better at the lower temperature.

Young cultures occur as Gram-negative, non-sporeforming rods, varying considerably in length, from 1μ to 4-5μ, and in form from short, straight rods to larger rods of irregular shape. Many V, Y, and T-shaped formations occur, while some of the longer rods show rudimentary budding. In such cultures may be also noted larger coccoid bodies, apparently similar to the "arthrospores" or "cystites" noted in cultures of *Arthrobacter globiforme* and related species (7), from which radiate germinating rods, usually three in number. Cultures rapidly undergo characteristic morphological changes, so that usually after three days cocci become predominant, generally Gram-negative, though in many instances both Gram-negative and Gram-positive forms may occur. The cocci vary somewhat in size according to the medium, from 0.6-0.7μ on soil extract agar and *YX* agar to 0.8-0.9μ in soil extract semisolid. They occur in pairs, irregular groups like staphylococci, and in chains of up to six to eight cells suggesting that they are formed by a process of fragmentation from the longer forms, many of which show irregular staining indicative of a stage preliminary to coccoid formation. Somewhat larger than average coccoid bodies, noted in most cultures, are considered to be identical with the cystites which give rise to rod forms referred to, by germination upon transfer to fresh substrate. Motility has not been observed.

Organism No. B89 – Arthrobacter pascens n. sp.

This organism, which synthesizes the growth factor, differs markedly from organism No. 88 in that it has simple nutritional requirements, being able to grow with an inorganic source of nitrogen and without added growth factors. It may therefore be cultivated on the widest variety of media.

Colonies on agar plates are round, entire, white, glistening, and translucent, with a diameter of 1-2 mm. Cultures on nutrient agar and nutrient gelatin are viscous, though less so with age, but are not viscous on soil extract agar or semisolid. Surface streak cultures on nutrient agar show abundant, flat, filiform, whitish-cream colored growth with a smooth, glistening surface with a coppery sheen, and with heavy growth in the water of condensation. On soil extract agar the growth is rather less profuse. Stab cultures in soil extract semisolid show abundant stratiform growth restricted to the top 5 mm. of the medium. In nutrient broth there is uniform turbidity, no pellicle, but a rather viscous white sediment, the reaction becoming alkaline. The organism grows well on an agar medium containing inorganic salts and glucose (Med. *B* agar); streak cultures show abundant, filiform, cream-colored growth with very smooth and glistening surface and are viscous.

In nutrient gelatin, stab cultures show first a whitish, viscous surface growth, followed by a slow liquefaction which becomes characteristically stratiform; the liquefied portion is cloudy with a slightly brownish sediment and an alkaline reaction. Nitrates are reduced to nitrites and starch is hydrolyzed; indole is not formed nor is hydrogen sulphide produced. In milk cultures there is clearing without coagulation with little change in reaction, the medium becoming very viscous, capable of being drawn into long threads; considerable white growth occurs at the surface. Growth of the organism is enhanced by the addition of glucose, sucrose, mannitol, and glycerol, but only slightly by lactose; however, only a small lowering of the pH occurs in their presence, and no gas production is evident. Cultures in appropriate media give negative methyl red and Voges-Proskauer tests. The temperature range for growth extends from approximately 8° C. to a maximum of 35° C., no growth occurring at 4° or at 37° C. The temperature optimum is approximately 20° C.

On transfer to fresh medium growth appears to be initiated through the germination of the "arthrospores" or "cystites" from which radiate usually two or more rod-shaped cells, and which are noted within 16 hr. incubation. At a somewhat later stage the culture usually appears as predominantly Gram-negative rods, non-sporeforming, varying in length from approximately 1μ to 4μ and showing some irregularity in shape, many cells being slightly curved or bent. Frequent V, Y, and T-shaped formations may be observed in such young cultures. On further incubation, usually within two days, the culture passes to the coccoid stage and may show the appearance of a staphylococcus with many pairs and short chains. The cocci, which are uniformly Gram-negative, may vary somewhat in size according to the medium, from 0.5-0.7μ on solid agar medium and 0.7 to 0.9μ in semisolid or liquid media, with occasional, somewhat larger, cells. Repeated observations suggest a growth cycle which, following transfer of the organism to fresh medium, commences with the germination of the larger coccoid bodies whereby usually two or more rod-shaped forms develop. After the appearance of the rods, which may reach varying lengths, become somewhat irregular in shape, and may themselves increase by fission, there follows a fairly rapid change to the coccoid stage, which appears to arise chiefly through a process of fragmentation of the rod forms. The coccoid stage then persists and is the characteristic form observed in cultures more than two – three days old. The origin of the cystites is not entirely clear but it is considered that they represent enlarged cocci or are formed as terminal swellings frequently noted on rod forms. Motility has not been noted.

Systematic Position of the Organisms

In spite of pronounced difference in the nutritional requirements of the two organisms, the similarities noted with respect to their morphological characteristics suggest a close taxonomic relationship. Both are considered to belong to the soil corynebacteria, a group of organisms whose classification is

a subject of much diversity of opinion in spite of the fact that they comprise an important proportion of the indigenous bacteria of soil. Attention was first called by Conn (6) to the existence of a bacterium, abundant in soil, which showed pronounced pleomorphism, in that it changed rapidly in culture from a distinct rod to a coccus form and showed, in the rod stage, morphological characteristics suggestive of *Corynebacterium*. Conn, however, refrained from assigning the organism to this genus in view of certain differences that distinguish it from the type species, *C. diphtheriae* and other corynebacteria of animal origin, preferring to name it *Bacterium globiforme*. Though Krassilnikov (11) regarded this organism as a species of *Mycobacterium*, Jensen (9) considered it, as well as related types from soil, as a species of *Corynebacterium*. Topping (26) considered that *Bacterium globiforme* would eventually be included among the corynebacteria, while Lochhead (12) expressed the view that *B. globiforme* represented a special group of the corynebacteria with distinctive cultural and physiological properties.

More recently Conn and Dimmick (7) have proposed a new genus, *Arthrobacter*, for the inclusion of soil types which, though related to *Corynebacterium*, were regarded as having characteristics sufficiently different from those of the type species to warrant generic distinction. The present classification of *B. globiforme*, as representative of the soil corynebacteria, whereby it is relegated in Bergey's Manual, 6th Edition (2) to an appendix to the genus *Corynebacterium*, while certain related soil forms are included in the genus proper, is manifestly unsatisfactory. Lochhead (13) considered that the proposal of Conn and Dimmick had much to commend it, by avoiding undue extension of the commonly held definition of *Corynebacterium* while providing for an important group of related forms. Furthermore Clark (5) expressed the opinion "that with the gradual extension of its generic limits to include a diversity of bacteria, *Corynebacterium* as currently defined has become an undesirable and unwieldy genus", and recommended that the group of species typified by *Bacterium globiforme* Conn should be recognized as constituting the genus *Arthrobacter* Conn and Dimmick. In this the present authors concur.

Though organism No. *B*89 falls well within the definition of *Arthrobacter* as given by Conn and Dimmick (7), organism No. 88, in view of its special vitamin requirements, differs with respect to its ability "to live on inorganic nitrogen without added growth accessory substances". It is felt, however, that the definition of *Arthrobacter* should be less exclusive with respect to the nutritional characteristics of the members of the genus, and that the generic description should be widened sufficiently to include species, such as No. 88, that conform with the morphological designation even though they may differ in nutritional demands. In this connection Lochhead and Thexton (18) have reported the occurrence in soil of various types of bacteria requiring vitamin B_{12} for growth with morphological appearance characteristic of soil corynebacteria. In all respects save their vitamin requirements these appear to fall within the genus *Arthrobacter*.

The species No. 88 and No. *B*89 appear to have been hitherto undescribed and are considered to be most appropriately assigned to the genus *Arthrobacter* Conn and Dimmick. Organism No. 88, requiring the growth factor in question, is herewith named *Arthrobacter terregens* n. sp. Organism No. *B*89, capable of synthesizing the growth factor, is named *Arthrobacter pascens* n. sp.

Acknowledgment

The authors are much indebted to Miss Winnifred Ryan for very effective technical assistance throughout the course of the studies reported.

References

1. Bardos, T. J., Bond, T. J., Humphreys, J., and Shive, W. Relationship of the folinic acid group and the *Leuconostoc citrovorum* factors. J. Am. Chem. Soc. 71 : 3852. 1949.
2. Breed, R. S., Murray, E. G. D., and Hitchens, A. P. Bergey's manual of determinative bacteriology. 6th ed. Williams & Wilkins Company, Baltimore, Md. 1948.
3. Broquist, H. P., Stokstad, E. L. R., Hoffmann, C. E., Belt, M., and Jukes, T. H. Some observations on growth factors required by *Leuconostoc citrovorum*. Proc. Soc. Exptl. Biol. Med. 71 : 549-552. 1949.
4. Burton, Margaret O. and Lochhead, A. G. Nutritional requirements of *Arthrobacter terregens*. Can. J. Botany. In press.
5. Clark, F. E. The generic classification of the soil corynebacteria. Intern. Bull. Bact. Nomenclature and Taxonomy, 2 : 45-56. 1952.
6. Conn, H. J. A type of bacteria abundant in productive soils, but apparently lacking in certain soils of low productivity. N.Y. State Agr. Expt. Sta. Tech. Bull. 138. 1928.
7. Conn, H. J. and Dimmick, I. Soil bacteria similar in morphology to *Mycobacterium* and *Corynebacterium*. J. Bact. 54 : 291-303. 1947.
8. Hendlin, D., Caswell, M. C., Peters, V. J., and Wood, T. R. The nature of the TJ factor for *Lactobacillus lactis* Dorner. J. Biol. Chem. 186 : 647-652. 1950.
9. Jensen, H. L. Studies on saprophytic mycobacteria and corynebacteria. Proc. Linnean Soc. N.S. Wales, 59 : 19-61. 1934.
10. Kline, L. and Barker, H. A. A new growth factor required by *Butyribacterium rettgeri*. J. Bact. 60 : 349-363. 1950.
11. Krassilnikov, N. A. Die Entwicklungsgeschichte der Bodenmykobakterien. Zentr. Bakt. Parasitenk. Abt. II, 90 : 428-434. 1934.
12. Lochhead, A. G. Qualitative studies of soil micro-organisms. III. Influence of plant growth on the character of the bacterial flora. Can. J. Research, C, 18 : 42-53. 1940.
13. Lochhead, A. G. Chromogenic bacteria related to *Bacterium globiforme*. J. Bact. 55 : 579-580. 1948.
14. Lochhead, A. G., Burton, Margaret O., and Thexton, R. H. A bacterial growth factor synthesized by a soil bacterium. Nature, 170 : 282. 1952.
15. Lochhead, A. G. and Chase, F. E. Bacterial growth factors in soil. Science, 96 : 387. 1942.
16. Lochhead, A. G. and Chase, F. E. Qualitative studies of soil microorganisms. V. Nutritional requirements of the predominant bacterial flora. Soil Sci. 55 : 185-195. 1943.
17. Lochhead, A. G. and Thexton, R. H. Vitamin B_{12} as a growth factor for soil bacteria. Nature, 167 : 1034. 1951.
18. Lochhead, A. G. and Thexton, R. H. Qualitative studies of soil microorganisms. X. Bacteria requiring vitamin B_{12} as growth factor. J. Bact. 63 : 219-226. 1952.
19. Peeler, H. T. and Norris, L. C. A growth-stimulating factor for *Lactobacillus leichmannii*. J. Biol. Chem. 188 : 75-82. 1951.
20. Sauberlich, H. E. Relationship of purines, folic acid, and other principles in the nutrition of *Leuconostoc citrovorum* 8081. Federation Proc. 8 : 247-248. 1949.
21. Sauberlich, H. E. and Baumann, C. A. A factor required for the growth of *Leuconostoc citrovorum*. J. Biol. Chem. 176 : 165-173. 1948.

22. SAUBERLICH, H. E. and BAUMANN, C. A. Further studies on the factor required by *Leuconostoc citrovorum* 8081. J. Biol. Chem. 181 : 871-877. 1949.

23. SHORB, M. S. Unidentified growth factors for *Lactobacillus lactis* in refined liver extracts. J. Biol. Chem. 169 : 455. 1947.

24. SNELL, E. E. Bacterial nutrition—chemical factors. Bacterial physiology, *edited by* C. E. Werkman and P. W. Wilson. Academic Press, Inc., New York. 1951.

25. SPRINCE, H. and WOOLLEY, D. W. Relationship of a new growth factor required by certain hemolytic streptococci to growth phenomena in other bacteria. J. Exptl. Med. 80 : 213-217. 1944.

26. TOPPING, L. E. The predominant micro-organisms in soils. I. Description and classification of the organisms. Zentr. Bakt. Parasitenk. Abt. II, 97 : 289-304. 1937.

27. WILLIAMS, W. L., HOFF-JØRGENSEN, E., and SNELL, E. E. Determination and properties of an unidentified growth factor required by *Lactobacillus bulgaricus*. J. Biol. Chem. 177 : 933-940. 1949.

28. WRIGHT, L. D. and SKEGGS, H. R. The growth factor requirements of certain streptococci. J. Bact. 48 : 117-118. 1944.

THE USE OF A PERCOLATION TECHNIQUE IN STUDYING ANTIBIOTIC PRODUCTION IN SOIL[1]

By I. L. Stevenson[2] and A. G. Lochhead[3]

Abstract

The adaptation of a percolation technique to the study of the production of antibiotics in soil is described. Antibiotic activity is determined by assays of a continuously circulating soil solution without disturbing the soil sample itself. The advantages of this technique over existing methods of study are discussed. Results are presented illustrating the production of antibiotics in sterile soil by a *Penicillium* sp. and two unidentified *Streptomyces* spp.

Introduction

A number of investigators have reported on the control of soil-borne plant pathogens by the incorporation of fresh organic matter into soil (5, 9, 10). It has been suggested that the rapid increase in soil microflora brought about by the addition of this material might create conditions unfavorable for the pathogens through competition for existing nutrients or by an increase in the proportion of organisms antagonistic to them. One phase of this microbial antagonism, the possible production of antibiotic substances in soil, has recently received considerable attention. Various workers (2, 3, 4) have been able to show the formation of these antibiotics in sterile soil and in some cases in partially-sterilized soils inoculated with antagonistic organisms. Grossbard's (4) results suggest that the presence of available carbohydrate is the prime requirement for antibiotic production in soil. It is significant that this parallel exists between the requirements essential for antibiotic production in soil and the controlling conditions for a number of root diseases.

Existing techniques for the study of antibiotic production by selected antagonists consist of inoculation of pots containing soil and organic addenda followed by incubation for a prescribed period. During or at the end of incubation the pots are sampled and the soils extracted and assayed for presence of antibiotics. Various methods have been used in the extraction and recovery of antibiotics from soil. Grossbard (4) followed antibiotic production by expressing the soil solution by centrifugation. Gregory *et al.* (3) describe a technique in which displacement of the soil solution with 95% alcohol is used. Other workers have relied upon direct extraction of the soil with organic solvents. The presence of antibiotic substances in the various extracts is determined by microbiological assay.

This paper reports on the adaptation of the percolation technique first described by Lees and Quastel (7) to the study of antibiotic production in soil. Briefly this new method consists of the automatic and intermittent perfusion of a solution through a column of soil. The activity of the selected antagonists inoculated into the soil column is calculated from periodic assay of the perfusing solution for the presence of antibiotic substances.

[1] *Manuscript received September 9, 1952.*
Contribution No. 347 from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa.
[2] *Assistant Bacteriologist.*
[3] *Chief, Division of Bacteriology and Dairy Research.*

Methods

The Audus-type percolator illustrated in Fig. 1 was used during this investigation. The units were manufactured from Pyrex glass in order to withstand heat sterilization. The rate of percolation is adjusted by a piece of 4 mm. I.D. tubing packed with cotton in place of the thermometer-tubing regulator formerly in use. The mechanical operation of this type of unit has been adequately described by Chase (1). The soil column contains 50 gm. of the 2 to 3 mm. fraction of air-dried soil. Solid organic material may be added and mixed with the soil if so desired and the soil perfused with distilled water. In the event that a water soluble carbohydrate is to be added, the soil is percolated with 100 ml. of a solution of this material held in the reservoir.

For experiments requiring sterile soil, the soil column is autoclaved for 45 min. at 15 lb. Following this initial sterilization the soil column is attached to the remainder of the apparatus and further autoclaved for 15 min. The percolate is sterilized separately and 100 ml. added aseptically through the sampling tube to the reservoir. Sterility of the apparatus is ensured throughout the experimental period by fitting a cotton filter to the sample tube. The flow regulator serves as a back filter when the vacuum is shut off.

Inoculation of the soil is accomplished by loosening the rubber stopper at the top of the soil column and introducing aseptically 1 ml. of a spore suspension of the particular fungus or actinomycete under study. Inoculum was prepared from sporulating cultures on solid media suspended in a 1 : 5000 dilution of 'Tween 20'. Prior to inoculation it has been a constant practice to start the units and allow the percolates to perfuse through the soil until it is completely wet. This ensures an even distribution of the inoculum throughout the soil column. Following sterilization and inoculation percolation is started and the percolate assayed daily for antibiotic activity. No attempt was made to regulate the temperature in these studies and units were subject to variations in room temperature during percolation.

To measure antibacterial potency of the percolates the cylinder–plate method of Schmidt and Moyer (11) was adopted. *Staphylococcus aureus* (BDR No. 490), being sensitive to all antibiotic producers used in this study, was selected as the test organism. Since the diameter of the zone of inhibition is not directly proportional to the antibiotic potency of the percolate, a system of evaluation based on arbitrary units of claviformin was used for purposes of comparison. A standard curve was prepared by measuring zones of inhibition produced by known dilutions of pure crystalline claviformin (1 mgm. crystalline claviformin (M.P. 111) $\approx$ 48 units). By using appropriate dilutions of the percolate, satisfactory evaluation of antibiotic potency could be made and expressed in terms of claviformin.

Results

Fig. 3 shows the production of an antibiotic substance by a *Penicillium* sp. (BDR No. 560), a known producer of claviformin (8). One milliliter of a spore suspension was inoculated into the column of sterile compost soil (pH

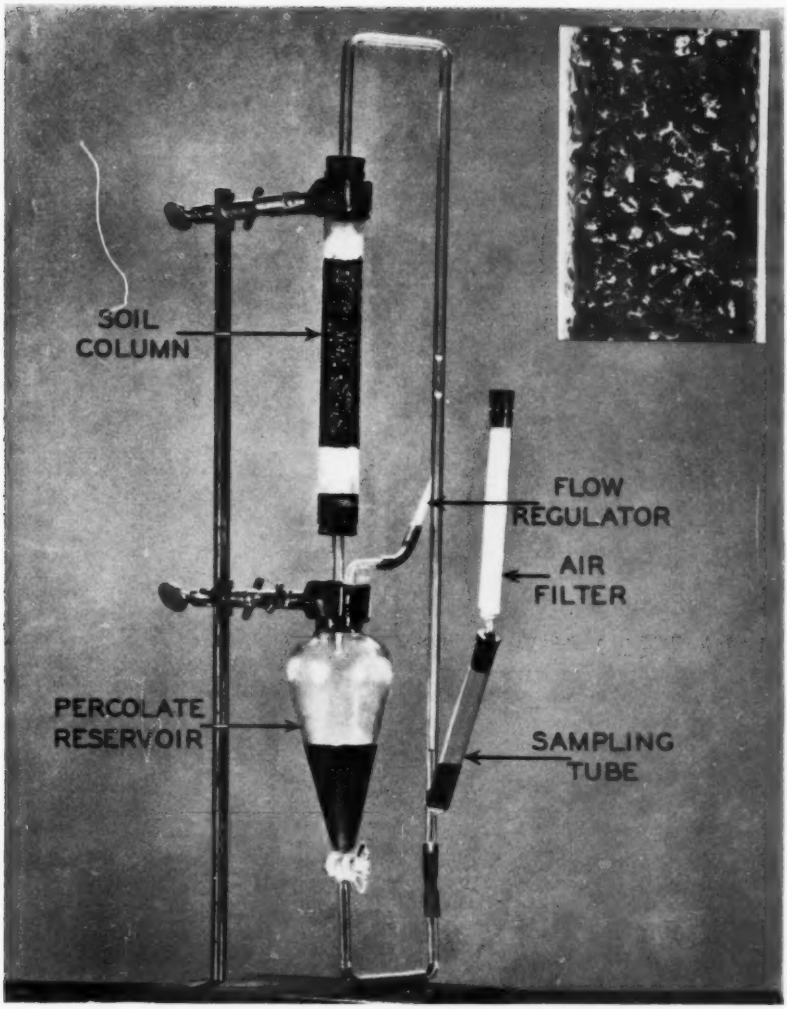


FIG. 1. Soil percolation apparatus.

FIG. 2 (Insert). Section of the soil column showing typical growth and distribution of the inoculated antagonist.

6.3) and the units percolated with 100 ml. of a 2.5% glucose solution (soil weight). Parallel formation of the antibiotic illustrated by the two solid-line curves representing duplicate units indicates the reproducibility of the method. Following initial detection of the antibiotic in the percolate daily assays were carried out. The results yielded a curve almost exponential in nature until peak of production is reached at eight to nine days. Beyond this peak there is a gradual decrease in antibacterial potency of the percolate. Sterile soil inoculated with *Penicillium* sp. (No. 560) and percolated with distilled water failed to show the production of an antibiotic.

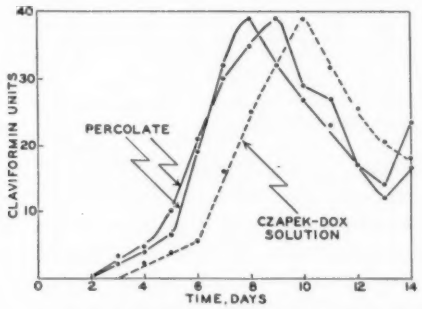


FIG. 3. Production of antibiotic by a *Penicillium* sp. in Czapek-Dox solution and in sterile soil percolated with glucose solution.

The suitability of soil as a medium for antibiotic production has often been questioned. Because of the very specific requirements for antibiotic formation, a certain medium may support luxuriant growth without the formation of the desired inhibitive agent. The broken-line curve in Fig. 3 represents the antibiotic production by the *Penicillium* sp. in a 2.5% glucose Czapek-Dox solution. The close agreement between the results of this test and those of percolation indicate the suitability of the latter technique in the study of antibiotic production in soil. Furthermore, provided a small supply of carbohydrate is present, soil appears to contain all the nutrients essential for production of the antibiotic.

The effect of green plant material on the production of an antibiotic by *Penicillium* sp. (No. 560) is illustrated in Fig. 4. In these units the four grasses were chopped and 3% by weight added to the soil column and percolated with distilled water. Typical growth and distribution of the *Penicillium* sp. in the soil column of the unit with added wheat are illustrated in Fig. 2. Growth generally becomes evident in the soil after about two days' percolation. Production of antibiotics in soil amended with green plant materials is considerably less than in units containing glucose. This might be expected in view of the comparative availabilities of the carbohydrate materials. Sporadic production of small quantities of antibiotics was often noted on percolation of soil amended with green plant material as evidenced by zones of incomplete inhibition.

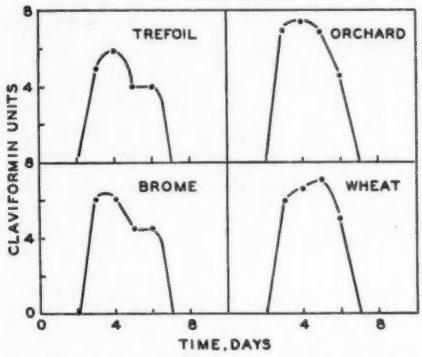


FIG. 4. Antibacterial activity of the percolate of soil inoculated with a *Penicillium* sp. and amended with green plant material.

Production of antibiotic by two *Streptomyces* spp., K1 and Y27, is illustrated in Fig. 5. Both organisms were isolated from soils of Northern Canada and exhibit *in vitro* inhibition of a number of plant pathogens (6). Curves shown in Fig. 5 represent the production of antibiotic in sterile soil percolated with a 2.5% glucose solution (soil weight). Both organisms exhibit a rapid increase in quantity of antibiotic until maximum potency is reached, followed by a gradual decline in activity. The streptomycete Y27 shows a considerably longer lag period than does strain K1.

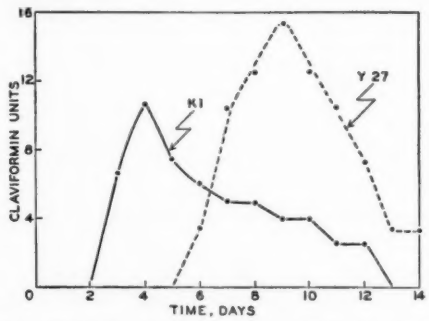


FIG. 5. Production of antibiotics by two *Streptomyces* spp. in sterile soil percolated with glucose solution.

Discussion

The Lees and Quastel percolation technique presents several advantages over existing methods for the study of antibiotic production in soil. Previously, assay of the soil for antibiotics could only be made through actual mechanical displacement of the soil solution. Equilibrium of the soil in the pots is upset by sampling and the number of samples obtained is naturally limited. In the percolation technique the production of antibiotic in soil is immediately

reflected in the composition of the reservoir fluid. Biological assay for antibiotics in the percolate can be used to follow the metabolic activity without disturbing the soil itself. Only an extremely small sample of the fluid (0.3 ml.) need be removed for assay. A much clearer picture of the actual course of antibiotic production can be obtained by this method. The water content of the soil is kept constant and maximal aeration is effected. The lack of uniformity of these factors in the pot technique has long been recognized as a disadvantage of this method in following biological processes.

The use of the technique is understandably limited to the study of those antagonists producing antibiotics which are neutral or acidic in nature. Basic antibiotics such as streptomycin are lost from the soil solution by rapid adsorption, often with a high degree of irreversibility, on the soil colloids. Much more severe extraction methods are required for the detection of these substances.

It is felt that this technique will be of value in establishing the effect of soil type, soil reaction, and different organic addenda on the production of antibiotics in soils by selected antagonists.

Acknowledgment

The authors are indebted to Mr. G. B. Landerkin for cultures of the two streptomycetes, and for supplying a quantity of crystalline claviformin.

References

1. CHASE, F. E. A preliminary report on the use of the Lees and Quastel soil perfusion technique in determining the nitrifying capacity of field soils. Sci. Agr. 28 : 315–320. 1948.
2. GOTTLIEB, D. and SIMINOFF, P. The production and role of antibiotics in soil. II. Chloromycetin. Phytopathology, 42 : 91–97. 1952.
3. GREGORY, K. F., ALLEN, O. N., RIKER, A. J., and PETERSON, W. H. Antibiotics as agents for the control of certain damping-off fungi. Am. J. Botany, 39 : 405–415. 1952.
4. GROSSBARD, ERNA. Antibiotic production by fungi on organic manures and in soil. J. Gen. Microbiol. 6 : 295–310. 1952.
5. HILDEBRAND, A. A. and WEST, P. M. Strawberry root rot in relation to microbiological changes induced in root rot soil by the incorporation of certain cover crops. Can. J. Research, C, 19 : 183–198. 1941.
6. LANDERKIN, G. B., SMITH, JANE R. G., and LOCHHEAD, A. G. A study of the antibiotic activity of actinomycetes from soils of northern Canada. Can. J. Research, C, 28 : 690–698. 1950.
7. LEES, H. and QUASTEL, J. H. Biochemistry of nitrification in soil. I. Kinetics of soil nitrification, as studied by a soil perfusion technique. Biochem. J. 40 : 803–815. 1946.
8. LOCHHEAD, A. G., CHASE, F. E., and LANDERKIN, G. B. Production of claviformin by soil penicillia. Can. J. Research, E, 24 : 1–9. 1946.
9. ROUATT, J. W. and ATKINSON, R. G. The effect of the incorporation of certain cover crops on the microbiological balance of potato scab infested soil. Can. J. Research, C, 28 : 140–152. 1950.
10. SANFORD, G. B. Soil borne diseases in relation to the microflora associated with various crops and soil amendments. Soil Sci. 61 : 9–21. 1946.
11. SCHMIDT, W. H. and MOYER, A. J. Penicillin. I. Methods of assay. J. Bact. 47 : 199–208. 1944.

PRODUCTION OF CYTASES ACTIVE ON BARLEY GUM BY BACTERIA OF THE GENUS *BACILLUS*[1]

By A. C. Blackwood

Abstract

One hundred and fourteen bacterial cultures representing most of the species in the *Bacillus* genus were tested for the production of extracellular barley gum cytase. Assays were made on shake-flask cultures grown on a medium containing glucose and yeast extract. Although all the organisms had some enzymatic activity, certain strains of *Bacillus subtilis* gave the best yields of cytase. On a medium with asparagine as the sole nitrogen source even higher yields were obtained. The crude cytase preparations were stable and after freeze-drying most of the original activity remained.

Introduction

It has been recognized for some time that hydrolysis of the plant cell wall is a key step in malting but few specific investigations have been reported. Preece (6) in a recent critical review outlines present knowledge and suggests a number of problems. Recently Preece, Ashworth, and Hunter (8) and Meredith, Bass, and Anderson (4) have published analytical data on the water soluble polysaccharides isolated from barley by several techniques. Both groups of workers have also made preliminary investigations of the action of various cytolytic enzymes on these substrates (1, 7).

At the suggestion of Dr. W. O. S. Meredith of the Grain Research Laboratory, Board of Grain Commissioners, Winnipeg, this survey was undertaken. He and his collaborators found (1) that a good source of enzyme was Wallerstein's "Alpha-amylase, special for analytical purposes" which is produced by a strain of *Bacillus subtilis*. The aim of the survey was to select cultures giving good yields of cytolytic enzymes and especially those that do not produce appreciable amounts of amylase concurrently.

Without definite knowledge of the chemical nature of these water soluble polysaccharides and of the specificity of the enzymes involved it is difficult to decide on the correct terminology for both the substrate and the enzymes (6). In this report the water soluble polysaccharides are referred to as 'gums' and the enzymes involved are called 'cytases'.

Materials and Methods

Cultures

A random selection of the *Bacillus* species available in our culture collection was made and 114 organisms were assayed. Eighty-four of these were cultures received previously from other laboratories and the rest were isolated and named in this laboratory.

[1] *Manuscript received September 15, 1952.*

Contribution from the National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Sask. Issued as Paper No. 144 on the Uses of Plant Products and as N.R.C. No. 2871.

The two cultures (*B62*, *B92*) giving the highest enzymatic activities were rechecked for their taxonomic position and both are *Bacillus subtilis* Cohn emend. Prazmowski. Both cultures are the Marburg type, i.e. there was no anaerobic growth on nutrient medium and no anaerobic gas production from nitrate medium.

Production of Enzyme

A preliminary trial with six cultures on three media containing respectively, as a carbohydrate source, glucose, starch, or barley gum showed that growth on a glucose medium gave relatively as good enzyme yields as either of the other substrates. Enzyme activity increased rapidly to a maximum (one to two days) and then remained constant. Thus for the survey 50 ml. of medium in a 500 ml. Erlenmeyer was inoculated with 2% of a 24 hr. inoculum, placed on a Gump rotary shaker revolving at 120 r.p.m., at 30° C. for three days. The medium contained 2% glucose and 0.5% yeast extract (Difco). Fermentation liquors were checked microscopically for contamination and the pH was determined. Enzymatic analyses were run on the clear supernate obtained by centrifugation of the culture liquor.

Enzyme Analyses

Cytase activities were estimated by measuring loss of viscosity of 2.5 ml. of gum substrate by adding 0.10 – 0.02 ml. of enzyme preparation. The substrate was prepared according to the scheme of Meredith, Bass, and Anderson (4) with a raw barley gum kindly supplied by Dr. Meredith. About 0.5% concentration of gum (the exact amount in each preparation was measured with the anthrone reagent (5)) gave an outflow time of 120 sec. at 30° C. from a 2-ml. jacketted pipette modified by the addition of 10 cm. of 0.8 mm. diameter bore capillary tubing in place of the regular tip. A fully liquefied sample had an outflow time of 13 sec. The gum substrate was buffered in all tests at pH 5.0 with 0.12 *M* acetate.

Results are expressed in arbitrarily defined enzyme activity units. One unit is the amount of enzyme necessary to reduce the viscosity (outflow time) of 1.0 mgm. of barley gum by one-half in 60 min., at pH 5.0 and at 30° C. The method of analysis and calculation of results is similar to that described by Landis and Redfern (3) for starch liquefaction by alpha-amylase. Wallerstein's alpha-amylase was used in preparing the reference enzyme.

The substrate is somewhat unstable and the viscosity falls gradually on standing as Meredith, Bass, and Anderson (4) previously have noted. This characteristic accompanied by the low solubility properties of the gum makes the preparation of a standard substrate difficult. A few trials showed, however, that the time required to reduce the viscosity to an arbitrary point varied inversely with the concentration of the enzyme. Also a comparison of several repeat analyses showed that the relative enzyme activity was constant for each of the preparations. Thus, although the analytical method was not too precise, ease and rapidity of measurement made it suitable for this survey.

Alpha-amylase was measured by estimating dextrinization activities where the enzyme activity unit is defined as the amount of enzyme necessary to hydrolyze 1.0 mgm. of standard starch substrate to an arbitrary end point in 60 min. at pH 5.0 and at 30° C. following a method used for bacterial amylase (2).

Results and Discussion

All of the bacteria showed some cytase activity and a few produced high yields (Table I). Repeat studies with the most active cultures from several species showed some variation but all still gave good yields of the enzyme. Further studies showed that the most active species, *B. subtilis*, contained two groups, in the first both cytase and amylase activities were high while in the second, amylase, though still present, was very low while the cytase yield was relatively high. Thus a representative from each group was chosen for further studies.

Preliminary lyophilization trials showed that the enzyme activity on a dry weight basis was lower than necessary because a considerable amount of the weight was added yeast extract. Therefore a study of production of enzyme in various inorganic media was made. Growth was good on all sources of inorganic nitrogen tested and although cytase was produced more slowly than on an organic nitrogen source final yields were high. The results obtained with four inorganic nitrogen sources are given in Table II where the levels of nitrogen were set at the optimum amount found for Ford strains of *Bacillus subtilis* in unpublished work by Dr. Neish of this Laboratory. Asparagine gave the best results.

TABLE I

RELATIVE CYTASE ACTIVITY OF 114 BACTERIA IN THE *Bacillus* GENUS

(Results are reported as the number of cultures found to produce the respective enzyme activity units per ml. of fermentation liquor)

Culture name	Number of cultures tested	Units of cytase per ml.				
		100	100-1000	1000-10,000	10,000-50,000	Over 50,000
B. subtilis	21	3	7	7	3	1
B. subtilis (Ford)*	39	10	19	8	2	
B. pumilus	5	4	1			
B. megatherium	5	3	2			
B. cereus	10	9	1			
B. alvei	10	1	8	1		
B. para-alvei	3	3				
B. circulans	5	3	1	1		
B. brevis	1	1				
B. coagulans	1	1				
B. polymyxa	9	4	5			
B. macerans	5	3	2			

* *Includes B. licheniformis strains.*

TABLE II

EFFECT OF VARIATION IN THE NITROGEN SOURCE ON ENZYME PRODUCTION

(The cultures were grown for five days on a medium containing nitrogen source, 2% glucose, 0.05% KH_2PO_4, 0.04% K_2HPO_4, 0.02% $MgSO_4 \cdot 7H_2O$, 0.005% $FeSO_4$, 0.02% NaCl, and 0.01% $CaCl_2$. The data given are the averages of two trials)

Culture	Nitrogen source	Enzyme activity, units per ml.	
		Cytase	Amylase
B. subtilis, B62	Yeast extract, 0.5%	49,000	
	NH_4 acetate, 1.0%	3900	1.6
	Urea, 0.5%	5500	0.8
	KNO_3, 0.2%	14,000	2.4
	Asparagine, 0.2%	16,000	2.3
B. subtilis, B92	Yeast extract, 0.5%	32,000	
	NH_4 acetate, 1.0%	1600	230
	Urea, 0.5%	5600	160
	KNO_3 0.2%	3700	100
	Asparagine, 0.2%	41,400	840

A number of samples were lyophilized with excellent recoveries of the enzyme and in Table III are recorded representative enzyme activities after freeze-drying. The data given here with yeast extract as a nitrogen source were obtained in a pilot plant run by fermenting 20 liters of broth in a 30 liter stainless steel fermentor for four days. The production of enzyme was slower than in shake flasks but the final yield was as high. In freeze-drying this large volume, some mechanical difficulties were encountered resulting in inactivation of some of the enzyme. The dried samples with asparagine as nitrogen source contain all the original activity.

The high enzyme activities found in these freeze-dried preparations is encouraging for future studies on these hydrolytic enzymes. It is possible with these two organisms to produce a product giving good amylase and cytase activity (as is found in Wallerstein's alpha-amylase) or to produce a

TABLE III

ENZYME ACTIVITY OF FREEZE-DRIED PREPARATIONS

Culture	Nitrogen source	Enzyme activity, units per mgm.	
		Cytase	Amylase
B. subtilis, B62	Yeast extract	1460	0.1
	Asparagine	1670	0.3
B. subtilis, B92	Yeast extract	780	20
	Asparagine	6400	270
Wallerstein's alpha amylase	——	4600	200

preparation having a good cytase activity with almost no amylolytic action. The widespread occurrence of cytases among the bacteria tried was rather unexpected. This may be partly explained as this gum consists mainly of polymerized glucose with lesser amounts of xylose and arabinose polymers (4) and is possibly more easily hydrolyzed than some other more complex carbohydrates. Some of the species other than *B. subtilis* (Marburg type) contain enough high enzyme yielding cultures to warrant further studies.

References

1. BASS, E. J., MEREDITH, W. O. S., and ANDERSON, J. A. Enzymes that degrade barley gums. 1. Isolation from a bacterial source. Cereal Chem. 29: 262-272. 1952.
2. BLACKWOOD, A. C. Ph.D. Thesis, University of Wisconsin, Madison, Wisconsin. 1949.
3. LANDIS, Q. and REDFERN, S. Methods for determination of alpha-amylase. III. Improved starch liquefying method. Cereal Chem. 24 : 157-166. 1947.
4. MEREDITH, W. O. S., BASS, E. J., and ANDERSON, J. A. Some characteristics of barley, malt and wort gums. Cereal Chem. 28 : 177-188. 1951.
5. MORRIS, D. L. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science, 107 : 254-255. 1948.
6. PREECE, I. A. The plant cell wall, cytolysis and malting. Wallerstein Labs. Communs. 11 : 119-133. 1948.
7. PREECE, I. A. and ASHWORTH, A. S. Cytolysis in germinating barley. II. Preliminary study of enzyme relationships. J. Inst. Brewing, 16 : 40-47. 1950.
8. PREECE, I. A., ASHWORTH, A. S., and HUNTER, A. D. Cytolysis in germinating barley. I. Some barley and malt polysaccharides. J. Inst. Brewing, 16 : 33-40. 1950.

THE RESPONSE OF POTATO TUBERS TO A PERIOD OF ANAEROBIOSIS

I. DRIFTS IN CARBON DIOXIDE PRODUCTION[1]

By Dorothy F. Forward[2]

Abstract

A survey of the course of carbon dioxide output of potato tubers upon transfer from air to nitrogen shows that the form of drift in nitrogen undergoes metamorphosis with the lapse of time between harvest and the anaerobic experience. A stable form is attained about three months from harvest.

On restoration of air after nitrogen a temporary outburst of carbon dioxide occurs, and both form and magnitude of this after-effect change with time elapsed after harvest. The magnitude is also influenced by the duration of anaerobiosis. At any season, increased time in nitrogen produces an increased after-effect of more than proportionate magnitude. The rate of carbon dioxide output in air after nitrogen ultimately becomes steady, but is not necessarily equivalent to that preceding anaerobiosis. For the space of about three months, subjecting a tuber to nitrogen for two days or more at 22° C. causes the ultimate rate of carbon dioxide output in air to remain well above that preceding anaerobiosis, while it bears a constant ratio to the final rate in nitrogen before air was restored. This permanent displacement of the rate in air is not at any time brought about by an anaerobic period of only one day.

The nature of the drifts in metabolic state of the tubers that underlie these metamorphic changes in response to a period of anaerobiosis is still under investigation.

Introduction

Potato tubers have long served as a classical example of plant organs that accumulate little or no alcohol in the absence of oxygen, and Barker and Saifi (3, 15, 16) have shown that lactic acid may be a major product of their fermentation. But they produce carbon dioxide anaerobically, the derivation of which is at present unknown. They are therefore a subject of interest to the student of the effects of anoxia on plant metabolism.

Several investigators have followed the course of carbon dioxide production by tubers in the absence of oxygen, and some diversity appears in the results. No one has made a sufficiently thorough attempt to determine whether the differences are reconcilable. Choudhury (11) transferred King Edward potatoes from air to nitrogen at 25° C., at unspecified seasons, and found a falling rate of NR* from the time of exclusion of oxygen until the establishment, one or two days later, of a depressed adjusted rate (parallel to the air line). Boswell and Whiting's (9) published example shows, at 29° C., a definite rise of NR above the air line, followed after nine hours by a decline below it. They state that the excess carbon dioxide above the air line was greatest with 'red' varieties in the autumn and winter, least with 'early'

[1] *Manuscript received September 22, 1952.*

Contribution from the Department of Botany, University of Toronto, Toronto, Ont. This study was carried out with the assistance of a grant in aid of research furnished by the University of Toronto.

[2] *Assistant Professor, Department of Botany.*

* *NR was introduced by Blackman (6) to denote the magnitude of carbon dioxide output in nitrogen. Abbreviations of this kind will be used here because of very frequent reference to this and similar quantities.*

varieties in April and May. Barker and Saifi (3) reported an initial increase in NR above the air line lasting for one to three days at 10° C., followed by a decline to a low level and a subsequent rise several days later. They obtained such records with King Edward potatoes stored at 10° C. until December, January, or March. Appleman and Brown (1) studied the anaerobic carbon dioxide production of three varieties after various storage treatments. Their determinations were at 24 hr. intervals, but their tables make it clear that in some experiments NR began at once to decline, while in others it rose above the air line, and in some instances remained above it for four days.

Boswell and Whiting attributed the initial excess of NR above the air line to a release of 'bound' carbon dioxide caused by a presumed increase in acidity of the tissues in the absence of oxygen, but Barker and Saifi measured the pH and found that it did not fall appreciably during the time of excess carbon dioxide output, and were convinced that the excess could not have been merely released from 'binding', but was of metabolic origin. This conclusion is supported by the variability of carbon dioxide output in nitrogen. For instance, in Appleman and Brown's experiments with McCormick potatoes the ratio of NR to the air line two weeks after harvest was close to 0.6 for three successive days, but with tubers of the same variety stored for a further three – nine weeks at or near room temperature it remained well above 1.0 for four days. Such different responses could hardly appear if the excess carbon dioxide were merely a physical result of changing the tubers from air to nitrogen.

Boysen-Jensen (10), Choudhury (11) and Appleman and Brown (1) have published 1/N,(i.e. intramolecular/normal) or NR/ALR*, ratios for potatoes. Boysen-Jensen's values were determined from (1) total carbon dioxide production in one – three days in hydrogen and (2) an average of rates in air before and after the anaerobic period. They varied from 0.44 – 1.1. Choudhury's values for King Edward potatoes at 25° C. ranged from 0.31 – 0.36. He stated that his ratios were determined from extrapolations of initial NR "obtained by the method of Blackman"; but actually he extrapolated neither INR* nor ANR* as Blackman (6) did. Had he done so his INR/ALR would have been close to 1.0 and ANR/ALR close to 0.4. Appleman and Brown's values of I/N for McCormick potatoes respiring at 22° C. ranged from 0.52 – 1.55; for Irish Cobbler 0.41 – 1.23; and for Russet Burbank 0.34 – 1.40. They stated that "the Pasteur effect was very pronounced in McCormick potatoes". But they allowed 18–24 hr. to elapse between giving nitrogen and beginning to measure NR, and not always did their ratios become constant before the experiment ended. Never did they make sufficiently frequent determinations to define the drift of NR closely. Consequently most of their ratios are for neither initial nor adjusted NR, and are of little value in interpreting metabolic processes.

* *INR, ANR are abbreviations for initial and adjusted rates of carbon dioxide output in nitrogen; ALR for the air line, or concurrent output in air.*

As the present author (13) pointed out, the interpretation of 1/N ratios is not always a simple matter, and this is especially true of potato tubers where the fermentation is as yet incompletely understood. Nevertheless the production of carbon dioxide in the absence of oxygen is one of the important indices to the nature of fermentation, and a thorough knowledge of it is an essential part of an analysis of the anaerobic metabolism of a tissue. Its variability in tubers of different metabolic condition renders it of particular interest in potatoes. When a study of the nature of fermentation in these organs was undertaken, therefore, carbon dioxide production during and after a period of anaerobiosis was first studied. It soon became clear that this was variable in a uniform stock of the variety chosen, and a definite metamorphic pattern appeared that is a reflection of an ontogenetic drift in metabolic state of the tubers during storage. A large number of experiments was undertaken to survey the progressive changes, and so to provide a means of recognizing the metabolic state of any tuber under experimentation. This is being followed by biochemical studies of concomitant changes in other metabolites, but the present paper is confined to an analysis of the drifts in carbon dioxide production.

Materials and Methods

Potatoes of the variety Sebago were obtained in 1941 and 1945 from Guelph, Ont., from the Ontario Agricultural College, and in 1945–1950 from Charlottetown, P.E.I., from the Dominion Laboratory of Plant Pathology. The latter were of foundation A disease-free stock. For several years this stock was also grown at the University of Toronto Holwood plots from tubers obtained each year from Charlottetown. Upon arrival of potatoes in the laboratory they were washed, blotted dry, and those of useful size selected, weighed individually, and wrapped in onion-skin paper. The tubers were then either used immediately for respiration experiments, or were stored in earthenware crocks at room temperature or, most frequently, in a refrigerator at a temperature that varied from 7 – 9° C., but is for convenience referred to as 8° C. In 1945 potatoes from Guelph were held in common storage until January 4, and were then transferred to Toronto and stored at 8° C.

For respiration measurements one tuber was sealed into a chamber consisting of a small bell jar on a ground-glass plate. Inlet and outlet tubes permitted a continuous flow of carbon-dioxide-free air, or nitrogen, through the chamber. All measurements of carbon dioxide output were made at 22.2° C. by an adaptation of the Pettenkofer method that provides for successive three-hour determinations throughout an experiment (Duff and Forward (12)). Rates of carbon dioxide output are calculated as mgm. per 100 gm. per hour. Except for Fig. 1, B they are computed to tuber weight at the beginning of a respiration experiment. At appropriate times the air stream was replaced for periods of one – three days by nitrogen freed of traces of oxygen by passage over heated copper wire.

Eighty-five experiments with individual whole tubers were included in the survey, in which at least one period of anaerobiosis was provided. These were distributed over the harvest years, 1941, 1945–7, 1949–50, and included potatoes grown in Guelph, in Prince Edward Island, and in Toronto.

Changes in Condition of the Tubers During Storage

During storage the potatoes lost weight slowly. The average rate of loss at 8° C. was 0.02% per day or 0.6% per month, while at room temperature it was about 9–10 times as great, or 5–6% per month. At 22.2° C. the rate of respiration throughout most of the storage period was close to 1 mgm. CO_2/100 gm./hr., which is equivalent to glucose consumption at the rate of about 0.5% of the tuber weight per month. This accounts for less than 10% of the 5–6% loss of weight at room temperature, which is therefore largely water loss. Similarly at 8° C., where both respiration and weight loss are slower, water loss must account for more than 80% of the decrease in weight, which, however, during a winter's storage did not exceed 5% of the original.

During respiration experiments the tubers lost little weight, not more than 0.5% of the original in 20 days. The average was 0.02% per day, which can be roughly accounted for by respiration of sugar. So water loss probably did not occur in the moist gas stream that flowed through the respiration chamber.

In storage at 8° C. sprouting usually began at the end of January or the beginning of February. A very few small sprouts were observed in February. More appeared in March and were 1–2.5 cm. long, and by late May or June they were numerous and reached a length of 5 cm. Sprouts were usually removed before a respiration experiment, so that the carbon dioxide production recorded is that of tuber tissue only; but in a few experiments they were left in place (cf. Fig. 1,B). On some occasions tubers that had not started to grow in storage did so during a long experiment at 22° C. This occurred in experiments started in December or January, or (in 1947) at the end of November. In the earlier experiments, even when 27 days in duration, sprouts did not exceed 5 mm. in length; in those of January they grew to 10–25 mm. in 20–26 days. In February to May but not in June, some regeneration occurred after initial removal of the sprouts, but the secondary growths were attenuated and minute.

Experimental Results

Initial Respiration Rate in Air at 22.2° C.

When tubers held at room temperature were placed in the water bath at 22.2° C. little change in respiration rate occurred, but when they were brought from 8° C. an initial increase in respiration rate, lasting usually about 10 hr. indicated a readjustment of metabolic rates. After this a steady drift in rate was established, and in order to arrive at an initial rate free from the effect of temperature change, this steady drift was extrapolated to the time of beginning measurements, as in Fig. 2.

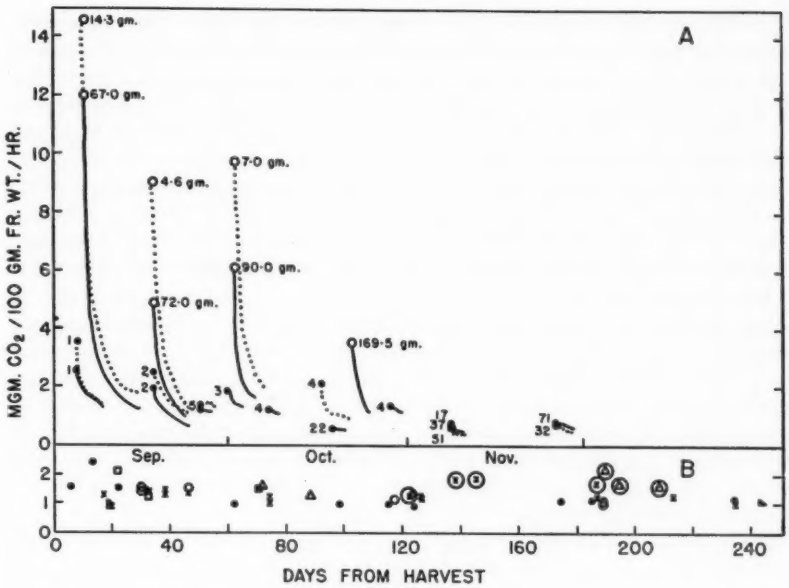


FIG. 1. Initial rate of respiration in air at 22.2° C.

A. Ordinate: rate of respiration. Abscissa: date of initial measurement. Large open circles represent initial rate of respiration on day of digging potatoes. Weights of tubers are marked. Dots represent initial rate after storage at room temperature. Numerals in front of the dots show the number of days elapsed between digging and measurement. Curves represent the drift in rate of respiration at 22.2° C.

B. Ordinate: Initial rate of respiration of tubers stored at 8° C. computed to weight at harvest. Abscissa: number of days elapsed between digging and measurement. Symbols indicate the harvest year:—1941 △, 1945 ✕, 1946 ●, 1947 □, 1949 ○, 1950 ◺. Circled symbols are for tubers with sprouts attached.

Respiration measurements were, in seven experiments, begun within two hours after digging the potatoes, and these provided an approximation to the rates at the time of detaching the tuber from the plant. Fig. 1, A shows (as open circles) initial rates of these seven potatoes, and indicates that as the tubers mature in September and October the respiration rate declines. The highest rates belong to groups of tubers weighing a few grams each*, but individual tubers increasing from 67 gm. on Sept. 5 to 169.5 gm. on Oct. 21 decline from 12 to 3.5 mgm./100 gm./hr.

Solid circles in Fig. 1, A show initial respiration rates of potatoes held for one or more days at room temperature before measurement began. A number beside each point indicates the number of days' delay. It is obvious that the initial rate is much lower than at the time of digging. The reason is apparent

* *A single tuber of this size did not produce enough carbon dioxide in three hours to give a reliable measure of its production rate with the method used, so several were placed in a respiration chamber and the average weight of a tuber is given. With these three exceptions all tubers were examined individually.*

from the early parts of the air drifts shown by lines that proceed from the open circles; for the rate declines with extreme rapidity from the time of digging, and within one – four days has reached values comparable with the initial rates determined only after an equivalent delay. Within days (10 for the youngest tubers, two or three for more mature ones), the rate has reached the same order of magnitude that is maintained throughout the winter. It is clear that even an immature tuber, detached from its parent plant, cannot maintain the relatively high rate of respiration that it has *in situ*, and soon subsides to the very sluggish state that is characteristic of potatoes in storage. This occurs in spite of the presence of an ample supply of reserve carbohydrate in the form of starch.

Fig. 1, B shows extrapolated initial respiration rates of potatoes that were stored at 8° C. for periods of from six days to eight months. Here the rates are plotted against storage time. Results obtained in six harvest years are combined, and, at least after the first 20 days, a high degree of uniformity is manifest. Variations in the early part of the season are associated with the degree of maturity of the tubers. The two highest initial rates belong to tubers that were dug about three weeks earlier than the rest, which were harvested at the normal time.

It should be stated that in some instances the exact day of harvest could not be ascertained, and the date adopted as the probable one may be in error by a few days. This could make little difference in the duration of long storage periods, but would be more important in defining short periods, and may be responsible for some of the scatter observed in the early portions of those graphs where various functions are plotted against storage time.

The drift in initial air respiration of stored potatoes, as shown in Fig. 1, B, is from about 1.5 to about 1 mgm./100 gm./hr., reached after approximately 100 days. After this time sprouts began to grow; but although intact sprouted tubers showed increased initial rates (circled points in Fig. 1, B), those from which the sprouts were removed continued throughout the remainder of an eight month period to respire at the rate of about 1 mgm./100 gm./hr. when placed at 22.2° C. One tuber held for 16 months at 8° C. had an initial rate at 22.2° of 2.08, while two held for 17 months had rates of 1.74 mgm./100 gm./hr. It therefore appears that the initial rate rises eventually, but this did not happen until more than eight months had elapsed.

The respiration rate of the sprouts themselves is, of course, much higher than that of tuber tissue. Sprouts 2–3 cm. long in March and April respired about 30 mgm./100 gm./hr., while the corresponding rate for the tubers from which they were detached was less than 1.

Respiration rates were usually computed to the current weight of the tuber, and, on this basis, initial rates rose slightly in late winter and spring. But it has been shown that the small loss of weight during storage is largely the result of water loss, and in order to avoid the appearance of change in rate caused by loss of water, the rates in Fig. 1, B were computed to the original weight at the time of harvest.

Respiration Drift in Air at 22.2° C.

Respiration rates were measured for several days in air, at least until a steady drift had been established. Fig. 1, A shows that the high rate characteristic of newly harvested tubers declines rapidly to values of the order of 1–2 mgm.CO_2/100 gm./hr.; while that of tubers long detached from the parent plants declines only relatively slowly. The drift in air of potatoes held at 8° C. was, with very few exceptions in the six years of experimentation, essentially a slow decline, often linear, often initially more rapid than later. In the majority of experiments it was still declining when nitrogen was substituted for air. The form with more rapid initial decline was characteristic of newly dug potatoes and those stored for short periods, the linear decline of those stored for longer times. In three years in which determinations were frequent the change to linear form appeared between late October and December. Fig. 2, shows air drifts of the two most frequently encountered types, excluding those of newly dug potatoes, which can be seen in Fig. 1, A. Only three individual tubers did not conform. Of these, two manifested a constant rate, and one an extremely gradual rise (0.0009 mgm./100 gm./hr./hr.).

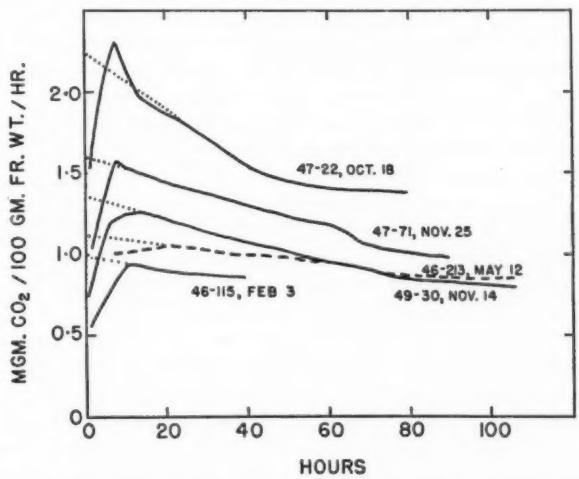


FIG. 2. Drift of respiration rate in air at 22.2° C. of potatoes previously stored at 8° C. Ordinate: respiration rate. Abscissa: hours at 22.2° C. In the identifying label on each curve the first number represents the harvest year, the second the time in days from digging. The date is that of starting measurement of respiration. Dotted lines are extrapolations (to obtain initial rate) of air drifts to the time of beginning measurements at 22.2° C.

When observation of the air drift was continued for long periods, the decline was sometimes found to persist for as long as 250–335 hr.; but on several occasions the decline came to an end after about 80, and on one or two occasions after 40–60 hr. The duration of the declining drift appears to depend on the properties of individual tubers, and not on the storage time.

Usually when the potato was transferred to an atmosphere of nitrogen the air drift was declining slightly, occasionally it was constant, and rarely inclining, as follows:

Number of experiments	Direction of slope of air drift	Magnitude of slope (mgm. CO_2/100 gm./hr./hr.)
49	Downward	0.001 – 0.004
18	—	0
9	Upward	0.004 – 0.003

Carbon Dioxide Output During a Three-day Anaerobic Period

After the air drift was established most tubers were transferred to nitrogen for one to three days and then returned to air. In the first year of experimentation it became clear that the form of drift in carbon dioxide output both during and after a period in nitrogen, was by no means always the same; and the experience of subsequent years established a definite sequence of forms, which will now be described. In most of the experiments the anaerobic period lasted for three days, and this group will be dealt with first.

Figs. 3–6 set forth the effects of a three-day period in nitrogen. The contrast in forms observed with a newly dug immature tuber and a stored mature tuber is strikingly apparent in Fig. 3. Several points of difference are noticeable. In the stored tuber record we observe: (1) a much lower rate in air; (2) a higher relative-to-air rate in nitrogen (although the full range of modification does not appear here); (3) a sharp reduction in the amount of carbon dioxide emitted immediately after return to air (the after-effect, or in abbreviated form, AE), together with a slower rise to and fall from the peak value; and (4) failure to approach the air line (ALR) when carbon dioxide production again attains a steady drift following the AE. In the September experiment the ratio of total carbon dioxide of the AE to the total NR was 2.2; in January it was 0.75. The AE is defined as the excess carbon dioxide above a line obtained by extrapolating the finally adjusted drift back to the time of return to air. The area included in AE in the January experiment is shaded in Fig. 3.

Figs. 4–6 give a more representative picture of the modifications encountered with stored tubers subjected to three-day anaerobic periods. These are essentially modifications in the pitch of carbon dioxide production in nitrogen as compared with air, in the sharpness and relative magnitude of the after effect, and in return or failure to return to the air line. The modifications are not haphazard, but constitute a definite metamorphosis. In Fig. 4 are representative records of potatoes held always at room temperature or 22.2° C. for periods of 5–75 days before placing in nitrogen; in Figs. 5 and 6 those of potatoes stored at 8° C. In the latter group the total period before the anaero-

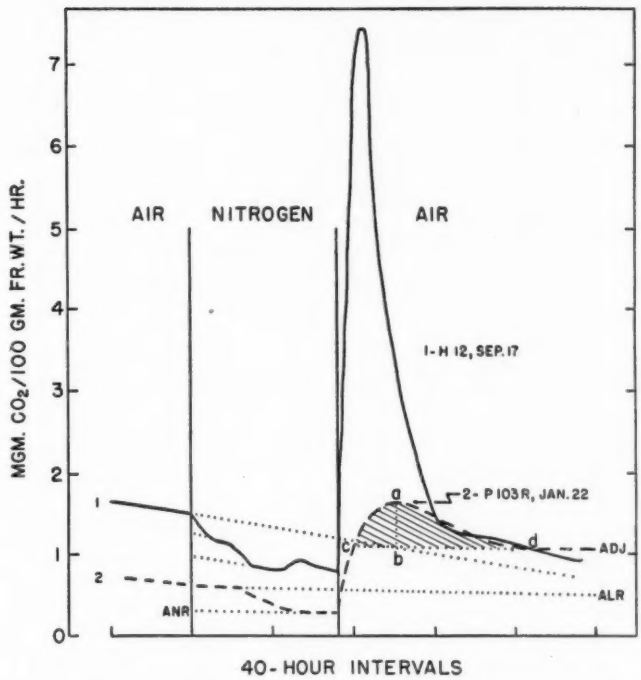


FIG. 3. Contrasting carbon dioxide records at 22.2° C. of immature newly harvested potatoes and a mature one stored at 8° C. until January. In the labels on this and succeeding figures the number of the curve appears first. H, P, and G denote potatoes grown in Holwood plots, in Prince Edward Island, and in Guelph, respectively. The next numeral signifies the number of days between harvest and the beginning of the anaerobic period, while the date of the latter follows. Where R follows the numeral it signifies storage in the refrigerator at 8° C.; where it does not appear storage was at room temperature.

Nitrogen replaced air at the time of the first upright; air was restored at the second. Curve 1, a solid line, is for three small tubers, of average weight 14.4 gm.; Curve 2, a broken line, is for one tuber, weight 162.3 gm., both of the 1946 harvest. Dotted lines are extrapolations of the air line (ALR), the adjusted rate in nitrogen (ANR), and the adjusted rate in air (ADJ) following the after-effect. The "steepness" of the after-effect in Curve 2 is equivalent to $\frac{ab}{cd} \times 100$ (see text).

bic experience (at 8° C. plus a few days in air at 22.2° C.) varied from 23 to 497 days. The rate of respiration in air at 22.2° C. was always measured for a few days before transfer to nitrogen, but only the last portion of the air drift is illustrated. The total storage time includes the preliminary period of respiration in air, which, for the sake of avoiding complexity, is not separately specified.

The nitrogen drifts encountered may be classified as belonging to three types which shift from one to the other so gradually as to permit the recognition of transitional forms. Type A, exemplified by Fig. 4, Curve 1, is

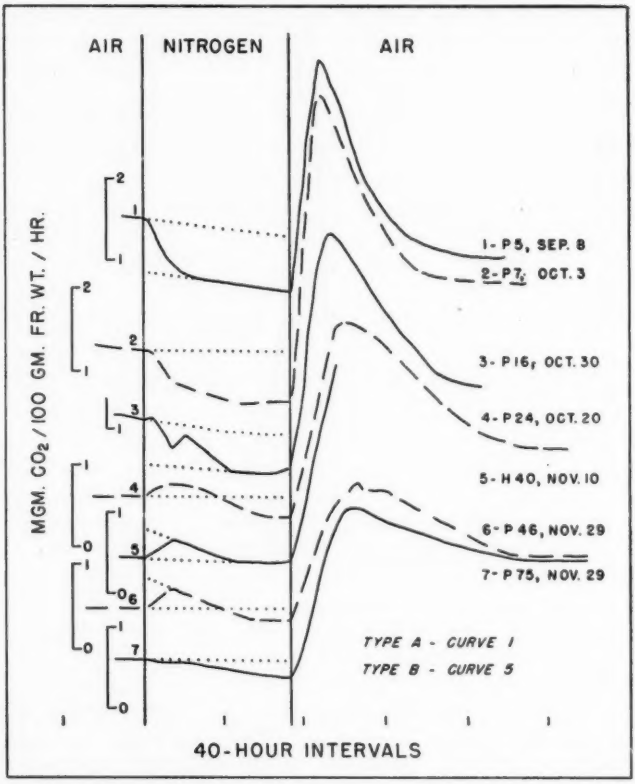


FIG. 4. Forms of carbon dioxide record at 22.2° C. during and after a three-day anaerobic period, for potatoes of the 1947 harvest held at room temperature for 5–75 days before transfer from air to nitrogen. For meaning of labels see subscript to Fig. 3. Each curve is the carbon dioxide record for one tuber. Dotted lines are extrapolations of the air line (ALR), 'initial' rate of carbon dioxide output in nitrogen (INR), and adjusted rate in nitrogen (ANR). Each curve has its own ordinate scale.

characterized by a rapid drop to a new line of drift below ALR. In about half of the Type A experiments the new drift was parallel* to ALR immediately after the transitory drop; in the others it first declined more rapidly, and a slight inflection initiated a parallel drift only after 20–45 hr., as in Curve 2. Such an inflection may also be seen in Curve 3, but a break in the downward drift appears here, and represents the beginning of a transition to Type B.

** An 'adjusted' drift in nitrogen would properly be one bearing a constant ratio to ALR, but in these experiments where ALR is usually declining slowly and where its position cannot be defined with perfect precision, it is difficult to distinguish between parallelism of NR and ALR and constancy of ratio between the two. It is more convenient to regard the adjusted NR as parallel to ALR.*

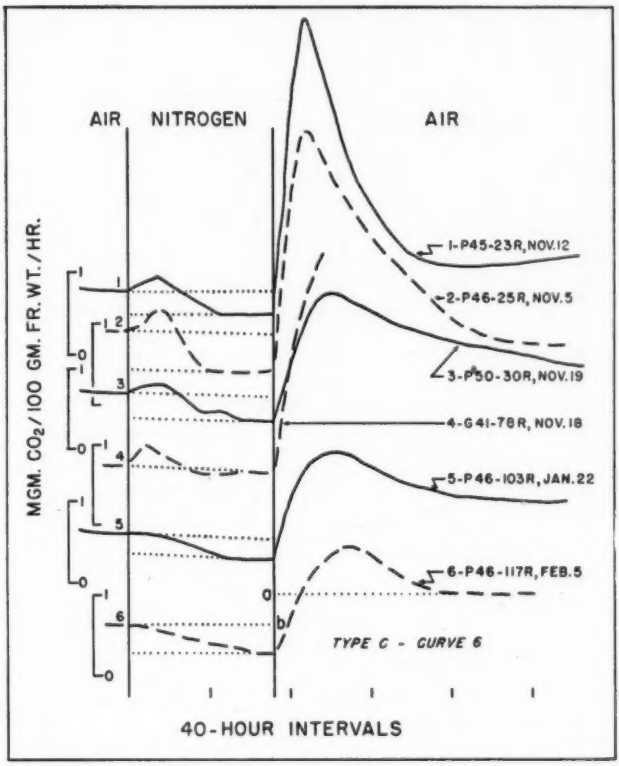


FIG. 5. Forms of carbon dioxide record at 22.2° C. during and after a three-day anaerobic period, for potatoes stored at 8° C., November to February. In the labels the first numeral following the letter denotes the harvest year, and the second the number of days from digging the tubers to transferring them to nitrogen. Otherwise the labelling is as in Figs. 3 and 4. Each curve is for one tuber, and has its own ordinate scale.

Another feature that appeared in several Type A records is illustrated in Curve 3. This is an upward inflection during the third day of anaerobiosis, which was usually sharp, and which in one or two extreme cases carried NR well above ALR. The upward inflection occurred in more than one-third of the Type A records and those between A and B, but not in Types B or C (except with two of the four potatoes examined after more than a year in storage). Barker and Saifi (3) found in their experiments at 10° C. an upward inflection after 8–10 days, and it is possible that it would have appeared more consistently here if the anaerobic period had continued longer. In Barker and Saifi's experiments it appeared to be associated with the accumulation of alcohol.

Type B, best exemplified by Fig. 4, Curve 5, begins with a rise above ALR, followed by a return to it in 24–48 hr. from the beginning of anaerobiosis, and

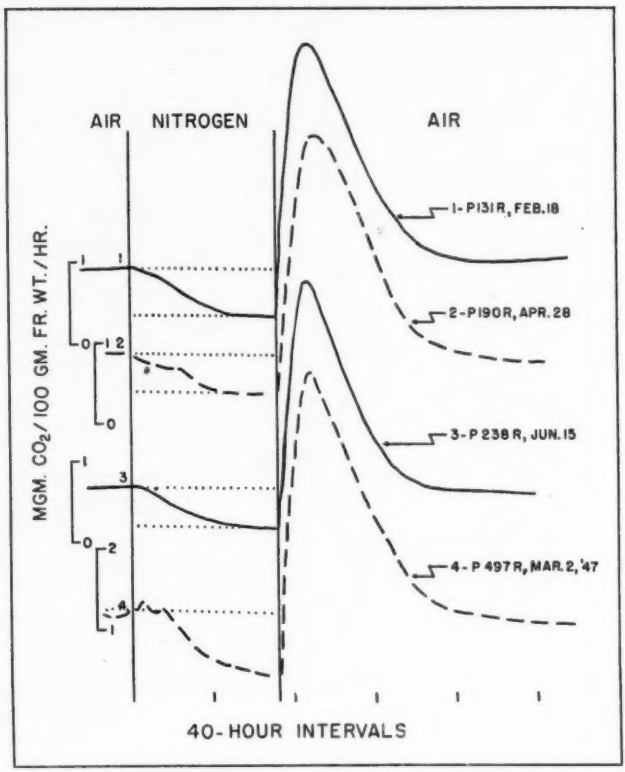


FIG. 6. Forms of carbon dioxide record at 22.2° C. during and after a three-day anaerobic period, for potatoes stored at 8° C., February to June, and the following March. All curves are for single tubers of the 1945 harvest, each with its own ordinate scale, and labelling as is in Fig. 3.

subsequent drift along ALR. Type C, involving a gradual decline from the time of entry into nitrogen appears in typical form in Fig. 5, Curve 6 and Fig. 6, Curves 1–3. Between the true types A and B or B and C lie a series of forms that are intermediate and represent transition from one to the other. In Fig. 4, Curves 3 and 4 show the A → B transition and Curves 6 and 7 are transitional between B and C. In Fig. 5, Curves 1–3 lie between A and B, 5 is between B and C, and 6 is the true Type C.

Type A was associated with a relatively high final respiration rate in air (FAR), just before the anaerobic period, viz. 1–2 mgm. CO^2/100 gm./hr., and a negative slope in the preceding air line. When FAR fell below 1.0 transition to Type B began. B was associated with FAR values of 0.4 to 1.1, C with 0.6 to 1.2, usually with negative, but occasionally with zero or positive ALR slope. The absolute rate of FAR was, with Types B and C,

not at all closely associated with the form of NR, which was, on the other hand, related to previous storage time. Type A is characteristic of newly harvested potatoes, whether small and immature in early September, or fully-grown in mid-October. It never appeared in typical form in the record of a tuber placed in nitrogen more than a fortnight after digging. Transition to Type B began in the records of tubers stored either at room temperature or at 8° C., usually about three weeks from harvest, in early November, and reached the type form in November or early December about 40–60 days after digging. Type B soon began to be modified toward C, and from January to June, 100 days or more from digging, Type C always appeared. Two tubers kept 17–18 months at 8° C. showed a slight rise above ALR before declining

TABLE I

FORM OF DRIFT OF CARBON DIOXIDE PRODUCTION IN A THREE-DAY ANAEROBIC PERIOD

Harvest year	Source of potatoes	Type of drift	Number of experiments	Date of anaerobic period	Days from harvest to anaerobic period	Illustration	Storage temperature, °C.
1941	Guelph	B	1	Sept. 26	17		8°
		B	1	Nov. 30	78	Fig. 5, Curve 4	8°
		C	3	Feb.–Apr.	128–196		8°
1945	P.E.I.	A→B*	1	Nov. 12	23	Fig. 5, Curve 1	8°
		B→C	1	Dec. 9	50		8°
		C	8	Feb.–June	131–254	Fig. 6, Curves 1–3	8°
	Guelph	C	2	Mar., June	149, 243		8°
1946	P.E.I. and Holwood	A	7	Sept.–Oct.	4–13	Fig. 3, Curve 1	Room temp.
		A	1	Nov. 2	10		8°
		A→B	2	Oct. 21, Nov. 5	18, 25	Fig. 5, Curve 2	8°
		B→C	1	Dec. 14	64		8°
		B→C	1	Jan. 22	103	Fig. 5, Curve 5	8°
		C	1	Jan. 22	103		8°
		C	4	Feb.–June	117–237	Fig. 5, Curve 6	8°
1947	P.E.I. and Holwood	A	9	Sept.–Oct.	4–8		Room temp.
		A→	1	Oct. 30	16	Fig. 4, Curve 3	"
		A→B	2	Nov. 10, Oct. 20	20, 24	Fig. 4, Curve 4	"
		B	3	Nov. 10	40, 54	Fig. 4, Curve 5	"
		B→	1	Nov. 29	46	Fig. 4, Curve 6	"
		→C	1	Nov. 29	75	Fig. 4, Curve 7	"
1947		A	1	Oct. 21	25		8°
		A→B	1	Nov. 29	46		8°
		→C	1	Nov. 29	75		8°
1949	P.E.I.	A→B	2	Nov. 20–30	36, 46		8°
		→C	1	Feb. 10	120		8°
		C	1	April 25	192		8°
1950	P.E.I.	A→B	2	Nov. 19	30	Fig. 5, Curve 3	8°
		C	1	June 28	251		8°

** An arrow preceding or following a single letter indicates a close approach to the type; an arrow between two letters implies a form intermediate between the two.*

in nitrogen, as in Fig. 6, Curve 4, but two others did not, so it is not clear whether Type C is maintained indefinitely. But it does remain until the end of a normal storage season.

This sequence could be recognized in each year of experimentation as may be seen in Table I, although experiments were not always so distributed as to discover all the stages. Type B, like A, was evanescent, and appeared in its typical form only when an experiment fell at the right time, but all years provided records indicating that the potatoes passed through this phase. The multiplicity of examples is sufficient to establish clearly that a definite metamorphosis occurs, and that a major feature of the ontogenetic development is a shifting of the level of the nitrogen drift in relation to ALR. The first change is an elevation changing Type A to Type B, soon followed by a lowering, when Type B gives way to Type C. But C is not a repetition of A. In Type A the rate of carbon dioxide production drops relatively rapidly, usually in six or nine hours, to a new line of drift that can be extrapolated to a zero-time value

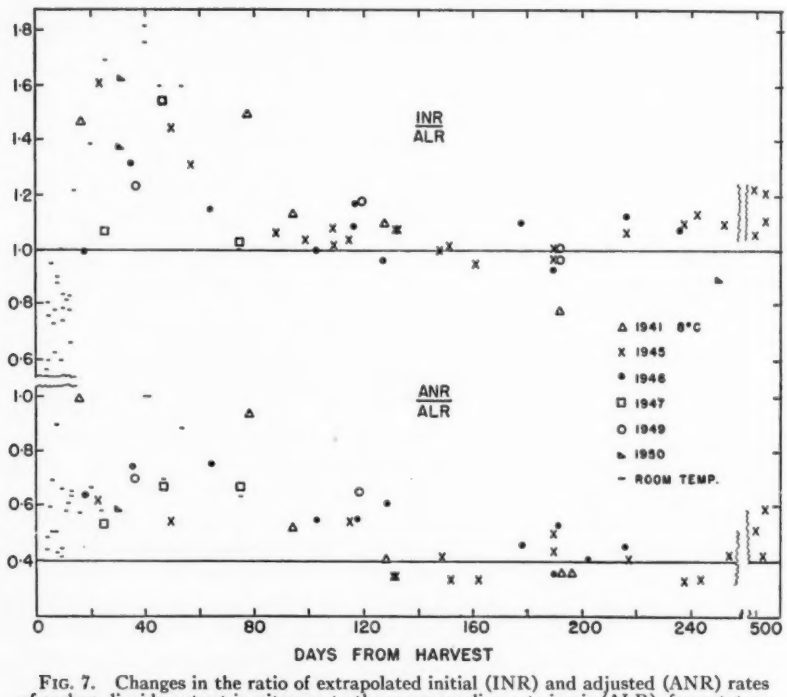


FIG. 7. Changes in the ratio of extrapolated initial (INR) and adjusted (ANR) rates of carbon dioxide output in nitrogen to the corresponding rate in air (ALR), for potatoes transferred from air to nitrogen for three days at different times after harvest.

Ordinate: ratio. Abscissa: days between harvesting the tubers and placing them in nitrogen. Small dashes are for tubers of both 1946 and 1947 harvests held at room temperature until used for experimentation. Other symbols are for potatoes stored at 8° C., as follows: 1941 Δ, 1945 x, 1946 •, 1947 □, 1949 ○, 1950 ◣.

well below that of the final rate in air (FAR), as in Fig. 4, Curve 1. In Type C the downward drift is gradual, lasting 30–60, usually 40–50 hr. Such a drift originates in a zero-time value nearly equal to FAR. Ultimately in Type C a drift parallel to ALR was nearly always established before the end of the 72-hr. anaerobic period and continued until the tuber was restored to air.

The degree of elevation of the nitrogen-drift for all experiments involving a three-day anaerobic period was evaluated by selecting two characteristics of the drift. The initial rate (INR) was obtained by extrapolating to zero time in nitrogen the first-established drift in nitrogen, omitting the relatively rapid shifts, upward or downward, that occur within the first 6–12 hr. The adjusted rate (ANR) was obtained by extrapolating the later established drift parallel to ALR. Such extrapolations appear as dotted lines in Figs. 3–6. The ratios of these two values to ALR give a measure of the initial and adjusted rates of carbon dioxide output in nitrogen as compared with concurrent rates in air, and provide a convenient means of describing the elevation of the nitrogen drift. These ratios are plotted in Fig. 7 against the time elapsed between digging the tuber and placing it in nitrogen.

It is clear that with few exceptions potatoes in all years and from all sources followed a recognizable trend. ANR/ALR was at first in the range of 0.4 to 0.6 and rose to a maximum value of 1.0. Examination of the small dashes in Fig. 7 will show that the maximum was obtained in 40 days by tubers held at room temperature, after which the ratio declined to 0.6 at 75 days, which is the longest storage period at this temperature. Symbols other than dashes in Fig. 7 represent tubers stored at 8° C. The increase in ANR/ALR was (with a single exception in 1941) slower than at room temperature, but within 80 days the ratio attained its maximum and then declined to about 0.4, where it remained for a period of about five months. After a total of 17–18 months in storage one of three potatoes still gave a ratio of 0.4 while two gave 0.51 and 0.61. The suggestion is that, once established at 0.4, ANR/ALR is constant for a very long time.

The same trends are recognizable in the ratio INR/ALR, which rises from values of 0.6–0.8, on digging, to 1.6–1.8; and then falls to 1.0 where it becomes stabilized. The time of reaching this stable value does not coincide with the establishment of constant ANR/ALR, but precedes it by some 50 days. After four months at 8° C. during which time a value of unity is maintained, a gradual rise again sets in. The primary rise in initial rate early in the storage history is, with tubers held at room temperature, contemporary with the rise in ANR/ALR; but with potatoes held at 8° C., the rise of initial rate precedes that of adjusted rate, and in fact is not delayed beyond that of tubers at room temperature. The effect upon the form of nitrogen drift of this separation in time between maximum initial and maximum adjusted rate can be seen by comparing Fig. 5, Curve 2 (INR high, ANR low, at 25 days) with Fig. 5, Curve 5 (INR near 1.0, ANR high, at 103 days).

Sprouting of the tubers did not affect INR/ALR. This ratio was already established at unity by 80 days from harvest, but sprouting did not begin until

about 110 days. The arrival of ANR/ALR at its constant value near 0.4 coincided approximately with the appearance of sprouts, but it is not yet clear whether there is any causal relationship.

Exceptional individuals are few. The greater scatter amongst early determinations of the two ratios is not surprising. Here the values are changing relatively rapidly, and slight variations in acceleration of the rate of change would displace individuals from the path of mean change. An error of two or three days in setting the harvest date (cf. p. 38) would also cause a small displacement along the abscissa axis, which would be important in a region of rapidly changing values. Furthermore, the early tubers varied greatly in size, degree of maturity, and actual harvest date, while those used later in the season were full grown and were dug at the normal harvest time. Indeed, the consistency of the data is remarkable, considering that the individual tubers represent six harvest years and three locations of culture, that they were dug at times ranging from the beginning of September to late October, and that they weighed from 5–200 gm. It indicates very definitely that time from harvest is a strong factor in determining the characteristics of the nitrogen drift.

The After-effect of a Three-day Anaerobic Period

When potatoes return to air after a period of anaerobiosis, an outburst of carbon dioxide carries its rate of production temporarily to a high value before a steady drift is again established. This was early observed by Singh (19). In the present experiments the ultimate drift is sometimes along ALR, sometimes well above it. The excess carbon dioxide above such a steady drift constitutes the after-effect, and is generally assumed to represent the oxidation of some substance or substances that accumulated in the absence of oxygen. Figs. 3–6 show that the after-effect, as well as the nitrogen drift, undergoes metamorphosis. It is subject to change in two respects: (1) its form, (2) the total amount of excess carbon dioxide relative to that evolved during the anaerobic period.

(1) *Changes in Form of the After-effect*

Fig. 3 illustrates the wide range in form of AE encountered. Of the two types shown here the very steep one is characteristic of immature potatoes placed in nitrogen within a few days of harvest; the more moderate one of tubers stored at 8° C. until mid-January. In Fig. 4 the obvious trend is a gradual flattening from the steep AE of September, five days after harvest, to the extended form shown at the end of November after 75 days' storage at room temperature. Fig. 5 shows the same trend with tubers stored at 8° C., the total time from harvest to the beginning of anaerobiosis being 23 days in Curve 1, 117 days in Curve 6; and the period of experimentation extending from early November to February. Fig. 6 shows that the flattened form was temporary, for later in the season a progressive steepening occurs, with a

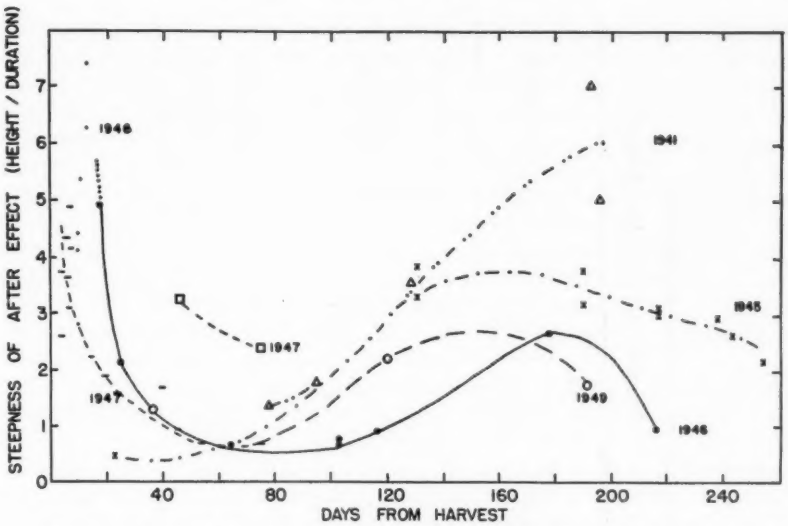


FIG. 8. Changes in steepness of the after-effect of an anaerobic period, for potatoes transferred to nitrogen for three days at different times after harvest.

Steepness $= 100 \times \frac{\text{height}}{\text{duration}}$ of after-effect.

Small dots are for 1946 potatoes, small dashes for 1947 potatoes held at room temperature; other symbols are for tubers stored at 8° C.:— 1941 △, 1945 x, 1946 ●, 1947 □, 1949 ○.

return to the form seen in the earliest curves of Figs. 4 and 5. The trends shown in Figs. 3–6 are essentially representative, but an attempt to show the variations in form in the whole series of experiments has been made.

Various indices have been examined. Probably the most satisfactory is the ratio between height of AE and its duration. Accordingly 'steepness' is defined as follows: Maximum rate of carbon dioxide production minus the extrapolated finally adjusted rate, divided by the duration in hours of the after-effect, the whole multiplied by 100, as a matter of convenience $\left(\frac{\text{Max. AE} - \text{ADJ}}{\text{Hr. of AE}} \times 100\right)$. For example, in Fig. 3, Curve 2, steepness $= 100\,\frac{ab}{cd}$. This function is plotted in Fig. 8* against the storage time. Other indices of the form of AE show similar distribution in storage time, e.g. actual maximum rate of carbon dioxide production, or acceleration to the maximum. The duration of AE, and the time required to reach the maximum show an inverse distribution.

The basic changes that appear in Fig. 8 are: first an early decrease in steepness to a minimum value, then an increase, and a subsequent decrease. Clearly,

** A few tubers showed signs of breakdown at the end of the experiment. These were excluded from Fig 8 and others dealing with the after-effect.*

TABLE II

FORM OF AFTER-EFFECT OF A THREE-DAY ANAEROBIC PERIOD WITH POTATOES KEPT AT ROOM TEMPERATURE

(Relationship between steepness of after-effect and degree of maturity, as judged by date of harvest and weight of tuber)

1946					1947					
Steepness*	Harvest date	Date of N.**	Tuber wt. (gm.)	Days: harvest to N.	Steepness*	Harvest date	Date of N.**	Tuber wt. (gm.)	Days: harvest to N.	Remarks
11.9	Sept. 5	Sept. 14	14***	12	4.9	Sept. 17	Sept. 23	46***	6	Immature
7.4	Sept. 20	Oct. 3	51	13	4.9	Sept. 15	Sept. 23	125	8	"
6.2	Sept. 20	Oct. 3	136	13	4.1	Sept. 5	Sept. 13	79	8	"
5.3	Sept. 5	Sept. 16	67	11	3.7	Sept. 3	Sept. 8	154	5	Mature (?)
4.5	Oct. 3	Oct. 13	95	10	3.6	Sept. 17	Sept. 23	72	6	Immature
2.8	Oct. 10	Oct. 21	166	10	3.1	Sept. 26	Oct. 3	179	7	Mature
					2.6	Oct. 21	Oct. 25	170	4	"
					2.2	Oct. 21	Oct. 30	147	9	"
					1.9	Oct. 21	Nov. 10	170	20	"
					1.7	Oct. 1	Nov. 10	68	40	Immature
					1.6	Sept. 26	Oct. 20	175	24	Mature (?)
					0.9	Oct. 21	Nov. 29	154	39	"
					0.7	Sept. 17	Nov. 10	84	54	Immature
					0.7	Sept. 15	Nov. 29	144	75	Mature (?)

* *Steepness* $= \frac{maximum\ AE - ADJ}{duration\ of\ AE\ in\ hr.} \times 100.$

** *N. signifies the beginning of the anaerobic period.*

*** *A group of small tubers was used for measurement of carbon dioxide output. The average weight is shown.*

however, factors other than the lapse of time after harvest affect the form of AE more than they do the form of nitrogen drift. One of these is the growing season. The actual magnitudes of steepness were different in different years, while the trends were similar. The steepest after-effects were found in September, 1946, but after 60–100 days very flat AE's appeared in that year, and the maximum observed later was lower than corresponding values in 1941 and 1945. In 1947 the early decrease in steepness of AE with potatoes at room temperature corresponded with that in 1946, but with two potatoes kept at 8° C. the decline was delayed. In 1941 two potatoes from Guelph had very steep AE's at 192 days, while one observed at 17 days showed virtually no after-effect. This absence of AE was unique but since only one tuber was investigated early in 1941 it is impossible to tell whether it was characteristic of that year.

Even within one harvest year there was a wides catter in values of steepness amongst the earliest experiments, which suggests the influence of still other factors at this time. The data in Table II indicate that degree of maturity is one of these. Experiments of 1946 and 1947 are set out in descending order of magnitude of steepness. Amongst those in which the anaerobic period began within a fortnight after harvest, there is a general association between declining steepness and increasing maturity, i.e. increasing tuber weight and lateness of harvest date. Correlation coefficients were determined between steepness of AE (x) and: tuber weight (y), harvest date (z), and lapse of time after harvest (t), as follows:

	1946 10–13 days from harvest	1947 6–9 days from harvest	1947 20–75 days from harvest
r_{xy}	$-.825$ ($P < .05$)	$-.668$ ($P < .10$)	Not significant
r_{xz}	$-.720$ ($P < .10$)	$-.736$ ($P < .05$)	Not significant
r_{xt}	Not significant	Not significant	$-.861$ ($P < .05$)

This analysis bears out the generalization that within the first fortnight steepness of AE is related to maturity of the tubers, but later to lapsed time after harvest.

The metamorphosis of the after-effect is not unrelated to that of the nitrogen-drift. In general, the early steep form (steepness more than 3.0) is associated with a Type A nitrogen drift, declining steepness (3.0 to 0.7) with transition through Type B, while the beginning of the secondary steepening coincides with the appearance of Type C.

It is plain from Figs. 3–6 that, while the form of AE is partially determined by the actual maximum rate of carbon dioxide production attained and by the acceleration and deceleration in rate, yet a major factor in producing the flat form is the failure to return to the previous line of drift in air. In Fig. 4,

Curve 1, the drift became adjusted, following the AE, at a level only slightly above ALR, but in Curve 7 the adjusted rate (ADJ) was nearly three times ALR.

The ratio of ADJ to ALR was determined for all the experiments that were continued until an adjusted rate was established. This was done by extrapolating both drifts to the time of restoration of the tuber to air after nitrogen, as in Fig. 5, Curve 6, where ADJ/ALR = a/b*. In some experiments this could be done without hesitation. In others, if the two extrapolations had to be made without further guidance it would be difficult to make them with sufficient certainty. This applies to those experiments in which the direction of drift was gradually changing with time. However, the adjusted NR served as a guide, and the assumption that the drifts are parallel both during and after the nitrogen period made it possible to find, for each drift, a line of extrapolation that could be accepted with reasonable certainty. In several instances concurrent experiments were performed, one of which provided an experimentally established ALR (e.g. Fig. 12). In these ADJ/ALR is clearly defined; in the others, although no high degree of precision is claimed, the ratios presented can be accepted as fair approximations.

ADJ/ALR is plotted in Fig. 9, B against the time elapsed between harvest and the beginning of the anaerobic period. The basic changes are: an early rise from 1.0 lasting 60–80 days, followed by a return to unity about 130

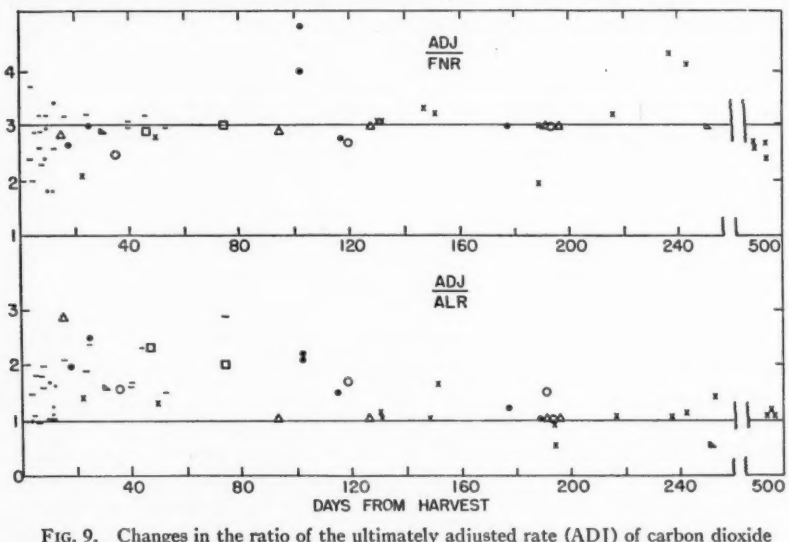


FIG. 9. Changes in the ratio of the ultimately adjusted rate (ADJ) of carbon dioxide output in air, after three days in nitrogen, to the final rate in nitrogen (FNR) and to the air line (ALR) extrapolated from the original drift in air before nitrogen. Symbols are as in Fig. 7 and 8.

* *The air line was determined for several days before nitrogen was given and its direction established, although this is not apparent in the illustration.*

days after harvest. The maximum values lie between 2.5 and 3.0, and coincide with the flattest form of AE. Ratios for five years and for all culture sites are included, and the consistency of values is sufficient to establish that there is a period of three or four months in which the adjusted rate of respiration following the after-effect does not return to the air line.

The ratio ADJ/ALR is not unrelated to the type of nitrogen drift, but the relationship is not a simple one. Low values within a fortnight of digging belong to Type A records. The general rise is associated with transition to Type B. High values occurred with both B and C, and by the time ADJ/ALR began to decline Type C was already established, and of course remained for the rest of the storage season. When Type C appeared high ANR/ALR ratios were associated with high ADJ/ALR, and they declined together. When ADJ/ALR became constant (130 days), ANR/ALR became established at about 0.4 (cf. Fig. 7).

The greatest scatter of points is, again, in the first fortnight after harvest. This is related to the variants of the Type A nitrogen drift. Here low values belong to those records in which NR remained, in the third day in nitrogen, parallel to ALR, while higher values belong to records where an upward inflection carried final NR higher than ANR. This directs us to an examination of the relationship between the *final* rate of carbon dioxide production in nitrogen (FNR) and the subsequently adjusted rate in air (ADJ), and we find that, unlike the other functions considered, ADJ/FNR is roughly constant throughout the storage season. This ratio was determined by extrapolating ADJ to the time of replacing nitrogen by air, and dividing the extrapolated value by the observed FNR. Fig. 9, A shows that at no time is there a basic trend away from the mean value of 3.0, although sporadic departures occur. The greatest scatter is at the beginning of the storage period, and only at this time do the values lie predominantly below 3. The lowest belong to immature tubers. The few exceptionally high values later in the season may be associated with physiological breakdown of the tissues, although none were included for tubers that showed visible signs of this. Obvious breakdown was accompanied by very high rates of carbon dioxide production in air after nitrogen.

The suggestion arising from the relative constancy of ADJ/FNR is that, during the three-day anaerobic period, some metabolic change occurs, at least in mature tubers, that determines the subsequent rate of carbon dioxide output in air, setting it at three times the final rate in nitrogen, whether or not this is equivalent to the previous air line. The reality of such a change in control is the more probable because, as will appear, it did not occur after only one day in nitrogen. Immature tubers appear to be more resistant to change.

(*2*) *Change in Relative Magnitude of the After-effect*

A further important aspect of the metamorphosis of AE is the change in total amount of carbon dioxide involved. Not only does the absolute amount undergo change, but also the ratio of carbon dioxide produced in the after-

effect to that produced during anaerobiosis (AE/NR). If the AE is to be regarded as the result of oxidation of a product or products accumulated in the absence of oxygen, then inconstancy of this ratio is important. Fig. 10 shows that, like other features of the AE, it was subject to change during storage of the tubers. The drift is similar to that of steepness, comprising

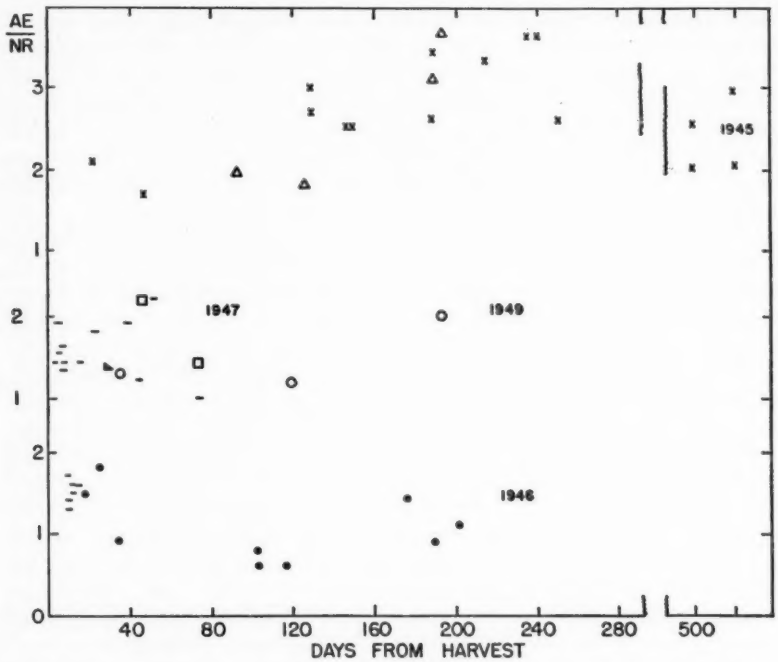


FIG. 10. Changes in the ratio of total carbon dioxide output during the after-effect (AE) to the total output in nitrogen (NR), for potatoes transferred to nitrogen for three days at different times after harvest. Symbols are as in Figs. 7–9. The ratios are plotted in three groups with displaced ordinate scales.

first a decrease in magnitude, then a secondary rise, and, at about 240 days, a subsequent decrease. Although the trends are common to all seasons, the magnitude differed in different years, and the ratios are plotted in three groups (1941 and 1945, 1947 and 1949, 1946) each with its own ordinate scale. In 1946 AE's were small in relation to total NR, in 1941 and 1945 they were large, and in the other years intermediate. The total range of magnitudes was from 0.6 to 3.7, the latter being six times the former. So, if the AE represents oxidation of substances accumulated during anaerobiosis, either the amount of these substances or the ability of the aerated tissues to oxidize them is subject to great change.

Carbon Dioxide Output During and After One- and Two-day Anaerobic Periods

Fig. 11 shows the effects upon carbon dioxide output of one day in nitrogen. Clearly the nitrogen drifts represent early portions of the types observed in three-day periods (Figs. 3–6). Type A appeared at five days, transition from A to B at 30 days, and Type C from 100 days onward. An experiment at seven days also gave Type A, and one at 88 days was intermediate between B and C. The form of after-effect also underwent the same metamorphosis as that observed after three days in nitrogen although the quantities of carbon dioxide involved were smaller. Not only was the absolute amount less, but also the amount relative to NR. The last is evident in Table III, where one can compare AE/NR for experiments involving potatoes of similar storage age kept for one-, two-, and three -day periods in nitrogen. The values for 1946—P117R and 1947—H5 are the only exceptions to the general observation that, when ratios for tubers of similar storage history are compared, they are found to increase substantially with increasing time in nitrogen. If the AE represents oxidation of products accumulated in the absence of oxygen, this can only mean that the rate of accumulation, relative to the output of carbon

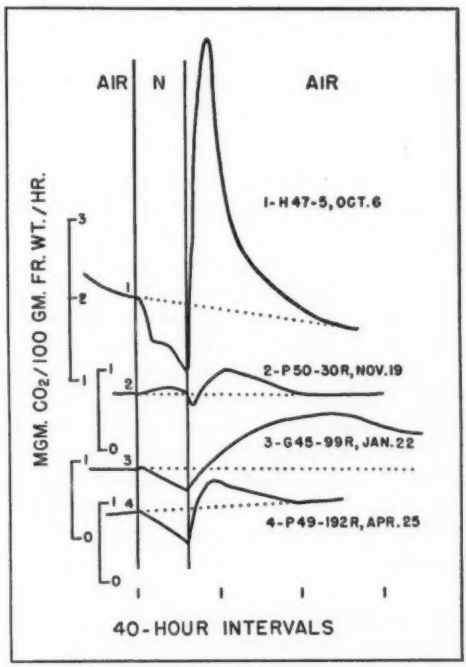


FIG. 11. Forms of carbon dioxide record at 22.2° C. during and after a one-day anaerobic period, for potatoes transferred to nitrogen at different times after harvest. Labelling is as in Figs. 3–6.

TABLE III

RATIO OF TOTAL CO_2 IN AFTER-EFFECT TO TOTAL CO_2 IN NITROGEN

Harvest year	History	Time in nitrogen		
		1 day	2 days	3 days
1945	G 110 R*	0.6		
		0.35		
	G 115 R		0.7	
	P 131 R			2.7
				3.0
	P 152 R			2.5
	G 161 R		1.75	
1946	P 117 R	0.5		0.6
	P 202 R			1.1
	P 237 R		0.5	
1947	H 5	1.5		
	H 6			1.4
				1.5
	P 7	0.9		1.3
	P 8			1.4
				1.6
1949	P 36 R			1.3
	P 46 R		1.0	
	P 192 R	0.4		1.9
1950	P 30 R	0.6		1.4

* *G, P, H, refer to culture in Guelph, Prince Edward Island, and Toronto (Holwood plots) respectively. The numeral shows the number of days between harvest and the beginning of the anaerobic period. R represents storage in the refrigerator at 8° C. Tubers not so marked were always at room temperature or 22.2° C.*

dioxide, increases on succeeding days of anaerobiosis. Singh (19) had already observed that the magnitude of the after-effect was greater the longer the exposure to nitrogen.

By referring again to Fig. 11 it may be seen that (with the possible exception of Curve 3, where it is not certain that an adjusted state was reached) the carbon dioxide drift following AE always returned to the air line, i.e. ADJ/ALR was always equal to 1.0. This was true whether the final NR was equal to ANR, or higher or lower, which means that ADJ/FNR was not constant, but varied from 0.93 to 2.2. This situation, where ADJ/ALR = 1.0 and ADJ/FNR varies, contrasts with that following a three day anaerobic period, where ADJ/ALR varied and ADJ/FNR approached 3.0. Although only nine such experiments with a one-day period were performed these were spread over the whole storage season September to April, 5 to 192 days, and used immature and mature tubers from all culture sites, stored at 8° C. and at room temperature.

The suggestion is that one day in nitrogen was not sufficient to bring about the metabolic change that leads at certain times to a failure of the adjusted respiration rate to return to the air line, and leads to a constant relationship to FNR. Two days in nitrogen was, however, long enough to establish the new relationship. Fig. 12, B provides the only illustration of the effect of a two-day anaerobic period, although similar observations were made several

times. Here concurrent records for two tubers are shown. The one provides a prolonged air line; the other is the record of a tuber placed in nitrogen for one day, and later for two days. After one day in nitrogen the carbon dioxide drift returned to the air line (ADJ/ALR = 1.0), and ADJ/FNR = 1.2; but after two days the adjusted rate was clearly above the air line (ADJ/ALR = 1.25), and ADJ/FNR = 3.0.

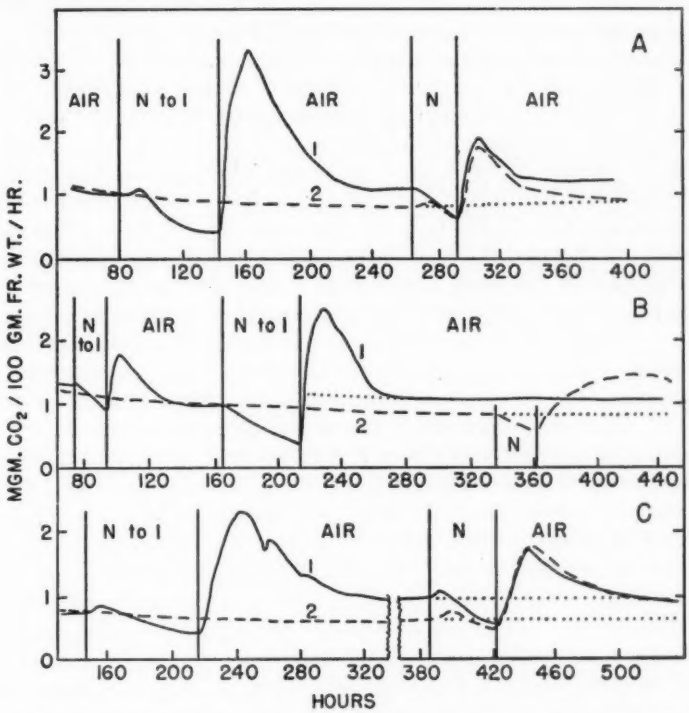


FIG. 12. Concurrent records of carbon dioxide output at 22.2° C. of pairs of tubers transferred separately to nitrogen for different periods of time.

Ordinate: rate of carbon dioxide output. Abscissa: time in hours from beginning measurements in air at 22.2° C. Uprights mark the time of beginning and ending periods in nitrogen for one or both tubers. Dotted lines are extrapolations of the air line and the ultimately adjusted rate after nitrogen.

A. Records of two tubers of the 1945 P.E.I. harvest. Measurements began Dec. 6, 1945, 52 days after digging. Tuber 1 (solid line) was in nitrogen for 63 hr. on the first and 30 hr. on the second occasion. Tuber 2 (broken line) was in air for 264 hr., then in nitrogen for 30 hr. simultaneously with 1.

B. Records of two tubers of the 1945 Guelph harvest. Measurements began Jan. 8, 1946, 85 days after digging. Tuber 1 (solid line) was in nitrogen for 24 hr. on the first occasion, 48 hr. on the second. Tuber 2 (broken line) was in air for 336 hr., then in nitrogen for 24 hr.

C. Records of two tubers of the 1949 P.E.I. harvest. Measurements began Nov. 14, 1949, 30 days after digging. Tuber 1 (solid line) was in nitrogen 72 hr. on the first occasion, 42 hr. on the second. Tuber 2 (broken line) was in air for 384 hr., then in nitrogen for 42 hr. simultaneously with 1.

Repeated Periods of Anaerobiosis

Frequently potatoes were subjected to further periods in nitrogen when a steady drift had been established after the first (Fig. 12). In four of these the first period was only one day in duration (e.g. Fig. 12, B), and the second was apparently unaltered by this short preliminary anaerobiosis, i.e. the carbon dioxide record matched that of the first anaerobic period of comparable tubers. But when the first nitrogen experience lasted for two or more days the record of the second was more advanced in metamorphic type, i.e. it resembled that typical of tubers stored for a longer time. For example, in Fig. 12A, Curve 2 of the NR at 264–294 hr., following air only, is intermediate between Types B and C, and resembles the first NR of Curve 1; but the concurrent NR Curve 1 (the second time in nitrogen) has progressed to Type C. Similarly in Fig. 12, C the second NR of Curve 1 has dropped in relation to the air line (ANR/ALR, 1st = 0.7, 2nd = 0.56) while in Curve 2, after air only, ANR /ALR = 0.7, as in the first NR of Curve 1. This means, of course, that the second NR of Curve 1 has progressed toward Type C. In 19 experiments of this kind, unless Type C was already established in the first period, the second nitrogen drift was more advanced in type. Evidently two, or three days in nitrogen hastened the normal metamorphosis but did not change it qualitatively. Somewhat meager evidence, not shown graphically, indicated that the metamorphosis of the after-effect was also accelerated.

Fig. 12, C illustrates the effect of a second anaerobic period on the subsequently adjusted drift. In Curve 1, ADJ remained well above the original air line after the first period in nitrogen (69 hr.), but after the second (42 hr.) there was no further change. Curve 2, on the other hand, now made the upward shift that had not previously appeared in this record of a single experience in nitrogen. Other experiments, not illustrated here, conformed. That is to say, after a *second* anaerobic period of two days, ADJ/ALR is typically unity, but ADJ/FNR is variable. But after a *first* anaerobic period of two days, as we have seen, ADJ/ALR may depart from unity, and ADJ/FNR is constant at 3.0*. Apparently, when once the metabolic change has occurred that makes ADJ independent of the previous ALR but links it to FNR, then a second anaerobic experience causes no further alteration.

One tuber experienced four successive anaerobic periods, at 25, 35, 47, and 57 days after harvest, with intervening aerobic periods to re-establish a steady carbon dioxide drift. The type of NR drift progressed from (A→B) in the first period, through B, and on to C in the fourth, and the after-effect became progressively flatter. After the first period in nitrogen ADJ returned to the air line, as in the records of a number of potatoes soon after harvest, but by the second it had advanced to the metabolic state in which ADJ remained well above ALR. The record of this tuber, therefore, passed through the metamor-

* *The only exception was that shown in Fig. 12, C where after 42 hr. in nitrogen ADJ/FNR = 2.0 and 2.2, which suggests that 42 hr. was not sufficient to complete the linkage of ADJ and FNR. A 30-hr. anaerobic period (Fig. 12, A) produces also a ratio below 3.*

phosis characteristic of potatoes at room temperature from about 25 to 75 days, which means that metamorphosis was accelerated by anaerobiosis.

Except for those few individuals which showed signs of breakdown, all but one conformed to this pattern. But the first experiment of 1941 was unique. Here ADJ/ALR after the first anaerobic period was nearly 3.0 which is typical, but after the second it was 0.4, and no after-effects intervened. This was the only occasion on which the air line after anaerobiosis was lower than before, and no explanation can be offered for this exceptional response. On the whole, repetition of anaerobic experience confirmed the general conclusions derived from a study of single periods in nitrogen.

General Discussion

A clearly defined ontogenetic drift in the characteristics of the anaerobic and postanaerobic carbon dioxide output of Sebago potatoes has emerged. When a tuber is transferred from air to an atmosphere of nitrogen a certain drift in rate of carbon dioxide output ensues, and the form of this drift changes with lapse of time between digging the tuber and placing it in nitrogen. The metamorphosis consists essentially in an elevation and then a depression of the basic relative-to-air rate, combined with a prolongation of the time required to establish an adjusted level. When a tuber is transferred from air to nitrogen almost immediately after harvest the rate of carbon dioxide production drops at once to an adjusted rate lower than that in air, while after storage for a short time the drop is accomplished in two stages rather than one. When a tuber enters nitrogen after still longer storage the basic level of carbon dioxide output is elevated until it is equivalent to the air line and the initial output is well above that in air. Still later in the storage season the level of the drift upon transfer to nitrogen gradually subsides again and carbon dioxide output is from the beginning less than in air but now it requires many hours to reach an adjusted rate. A stable form of drift with minimum adjusted rate is attained about three months after harvest and remains for the rest of a normal storage season.

When a tuber is restored to air after a period of one to three days in nitrogen, a temporary outburst of carbon dioxide intervenes before the output becomes steady. Such an after-effect of nitrogen, it is generally assumed, is the result of oxidation of accumulated products of anaerobiosis, and it also undergoes metamorphosis with increasing time after harvest. Its changes in form and magnitude presumably depend upon changes in the relative amounts of metabolites accumulated in the absence of oxygen and the pace of their removal when air is admitted, as well as upon departure from the air line of the ultimately adjusted rate of carbon dioxide production, which may at certain times follow a sufficiently long anaerobic period. Since the only function of the postanaerobic period that approaches constancy throughout the storage history of the tubers is the ratio of ultimately adjusted rate in air to final anaerobic rate of carbon dioxide output, some interdependence between these is suggested, whether direct or indirect; but its nature is as yet unexplained.

The classical series of experiments showing the effect of ontogeny on carbon dioxide output in relation to oxygen supply is that of Blackman and Parija (6, 7, 8, 14) on ripening apples. Their studies included the observation and analysis of changing drifts of carbon dioxide output of apples placed in nitrogen after various times in storage. Some at least of the types of nitrogen drift observed by the present author have been encountered by others using potato tubers and have been used as a criterion of the existence of the Pasteur effect or of oxidative anabolism. Low I/N ratios were interpreted by Choudhury (11) as meaning that potatoes have no oxidative anabolism. High ratios were regarded by Appleman and Brown (1) as evidence of a strong Pasteur effect and of oxidative anabolism. With Sebago potatoes not only do both high and low values occur, but there is an orderly pattern of drift from one to the other. Evidently there is a gradual readjustment of balance in metabolic processes during several months after harvest of the tubers, but our knowledge of its nature is still too incomplete to justify use of an I/N ratio as a criterion of the extent of the Pasteur effect in these organs. Barker and Saifi (3) reported that in King Edward potatoes "the occurrence of a Pasteur effect has been demonstrated for metabolic states in which the CO_2-production decreased immediately in nitrogen". This demonstration rested not on observation of carbon dioxide output alone but on analysis of accumulation of various intermediate and end products, which gave a measure of carbon loss in nitrogen and in air.

Wallerstein *et al.* (21) reported changes in the predominance of certain terminal oxidase systems in slices cut from tubers that were stored for different periods of time, and so have resolved some apparent inconsistencies. Schade and Levy (18) found that the time of washing slices also affected the balance of terminal oxidase systems, and indeed it is not surprising that a tissue which, undisturbed, is so mutable, will change its character when sliced and washed. Stoklasa (20) found that peeled potatoes, in contrast to the whole tubers used by others, accumulated substantial amounts of alcohol during anaerobiosis. Such observations bespeak caution in applying to the intact organ results obtained with slices.

A complete metabolic interpretation of the carbon dioxide drifts described here must await a better knowledge of the internal changes accompanying them. That the anaerobic metabolism of potato tubers is complex is clear from the work of Barker and Saifi (3, 4, 5, 15, 16, 17). They have found that in at least one metabolic state tubers accumulate considerable quantities of lactic acid and of a second, unidentified alcohol-soluble portion, while alcohol does not appear until later. In the meantime, sucrose disappears. In the postanaerobic period, they accounted for part of the after-effect as oxidation of lactic acid together with a temporary increase in sucrose concentration*, while the alcohol-soluble portion did not disappear. But many features

* *Barker (2) had previously shown that respiration rate in potatoes is dependent on sucrose concentration.*

remain to be further investigated, and the underlying reasons for the metamorphic changes described here are by no means clear as yet. Further studies are in progress and will be reported in due course.

In the meantime it is amply clear that the investigator who studies any phase of the metabolism of potatoes will greatly enhance the value of his work by defining the metabolic state of his tubers in so far as this is possible. At least the date of experimentation and the storage history should be stated. The carbon dioxide record provides one readily accessible index to the metabolic state, and its vicissitudes make it a valuable one.

Acknowledgments

The author acknowledges with gratitude the invaluable assistance of Miss J. Scorgie and Mrs. P. Richardson, who made most of the numerous determinations of carbon dioxide output that were necessary for these studies, and who helped also in the preparation of data for publication. She wishes to express her thanks to Dr. J. H. L. Truscott, Mr. H. N. Racicot, Mr. R. R. Hurst, and Mr. L. C. Callbeck for their helpfulness in providing suitable supplies of potatoes for experimentation; and to Dr. J. Barker for making available manuscripts of papers that are still in the press and for reading and criticizing the manuscript of the present paper.

References

1. Appleman, C. O. and Brown, R. G. Relation of anaerobic to aerobic respiration in some storage organs with special reference to the Pasteur effect in higher plants. Am. J. Botany, 33 : 170–181. 1946.
2. Barker, J. Analytical studies in plant respiration. VI. The relation of the respiration of potatoes to the concentration of sugars and to the accumulation of a depressant at low temperatures: Part 3—The relation of the respiration to the concentration of sucrose, Proc. Roy. Soc. (London), B, 119 : 453–473. 1936.
3. Barker, J. and Saifi, A. F. el. Studies in the respiratory and carbohydrate metabolism of plant tissues. I. Experimental studies of the formation of carbon dioxide, lactic acid and other products in potato tubers under anaerobic conditions. Proc. Roy. Soc. (London), B, 140: 362–385. 1952.
4. Barker, J. and Saifi, A. F. el. Studies in the respiratory and carbohydrate metabolism of plant tissues. II. Interrelationship between the rates of carbon dioxide production, of lactic acid production and of alcohol production in potato tubers under anaerobic conditions. Proc. Roy. Soc. (London), B, 140: 385–403. 1952.
5. Barker, J. and Saifi, A. F. el. Studies in the respiratory and carbohydrate metabolism of plant tissues. IV. The relation between the rate of carbon dioxide production in potato tubers in air following anaerobic conditions, and the accompanying changes in lactic acid content and sugar concentration. In press.
6. Blackman, F. F. Analytical studies in plant respiration. III. Formulation of a catalytic system for the respiration of apples and its relation to oxygen. Proc. Roy. Soc. (London), B, 103 : 491–523. 1928.
7. Blackman, F. F. Analytic studies in plant respiration. VI. Cambridge University Press. 1953. (In press.)
8. Blackman F. F. and Parija, P. Analytical studies in plant respiration. I. The respiration of a population of senescent respiring apples. Proc. Roy. Soc. (London), B, 103 : 412–445. 1928.
9. Boswell, J. G. and Whiting, G. C. Observations on the anaerobic respiration of potato tubers. Ann. Botany, 4 (n.s.) : 257–268. 1940.
10. Boysen-Jensen, P. Studien uber den genetischen zusammenhang zwischen der normalen und intramolekularen Atmung der Pflanzen. Kgl. Danske Videnskab. Selskab. Biol. Medd. 4 : 1–34. 1923.

11. CHOUDHURY, J. K. Researches on plant respiration. V. On the respiration of some storage organs in different oxygen concentrations. Proc. Roy. Soc. (London), B, 127 : 238–257. 1939.

12. DUFF, G. H. and FORWARD, D. F. Physiological and biochemical studies in plant metabolism. I. The respiration of the seedling wheat leaf in starvation and ontogeny. Can. J. Research, C, 27 : 125–145. 1949.

13. FORWARD, D. F. The respiration of barley seedlings in relation to oxygen supply. II. A metabolic interpretation. New Phytologist, 50 : 325–356. 1952.

14. PARIJA, P. Analytical studies in plant respiration. II. The respiration of apples in nitrogen and its relation to respiration in air. Proc. Roy. Soc. (London), B, 103 : 446–490. 1928.

15. SAIFI, A. F. el. Respiratory metabolism of potatoes under anaerobic conditions. Dissertation for the Ph.D. degree, University of Cambridge (University library). 1940.

16. SAIFI, A. F. el and BARKER, J. Respiratory metabolism of potatoes under anaerobic conditions. Rept. of the Food Investigation Board (Great Britain) for the year 1939. London. 1949. pp. 84–87.

17. SAIFI, A. F. el and BARKER, J. Studies in the respiratory and carbohydrate metabolism of plant tissues. III. Experimental studies of the formation of carbon dioxide and of the changes in lactic acid and other products in potato tubers in air following anaerobic conditions. In press.

18. SCHADE, A. L. and LEVY, H. Studies in the respiration of the white potato. III. Changes in the terminal oxidase pattern of potato tissue associated with time of suspension in water. Arch. Biochem. 20 : 211–219. 1949.

19. SINGH, S. B. Studies on the mechanism of respiration of massive plant tissues which offer great resistance to the passage of gases. Dissertation for the Ph.D. degree, University of Cambridge (University library). 1927.

20. STOKLASA, J. Der Kohlehydratumsatz in der Pflanzenzelle. Ber. Deut. botan. Ges. 44 : 248–262. 1926.

21. WALLERSTEIN, J. S., REINHART, R., LEVY, H., and SCHADE, A. L. Changes in metabolic characteristics of white potatoes as affected by conditions of storage. Food Research, 15 : 192–199. 1950.

FURTHER OBSERVATIONS ON THE REDUCTION OF FUSIFORM CAMBIAL CELLS IN *THUJA OCCIDENTALIS* L.[1]

By M. W. BANNAN[2]

Abstract

The multiplication of fusiform initials in the cambium is accompanied by extensive loss or transformation of these cells. A few of the failing cambial cells lapse into maturation quickly, but the majority are transversely subdivided with varying proportions of the segments surviving and undergoing ultimate conversion to ray initials. The loss or conversion is attended with reduction in cell size. Increase in cell volume lags behind cell division during the periclinal divisions of the transitional period. The tangential dimensions of the successively formed cells are continuously reduced, and sometimes radial expansion is also retarded, especially toward the cell tips. Simultaneous shortening of the cells is due to alteration in cell shape combined with asymmetry in periclinal division such that daughter cells of unequal lengths are produced. Repetition of the process, the smaller cell functioning as the initiating cambial cell in each instance, results in continued shortening.

Introduction

During the growing season the cambial zone is the site of extensive changes in cell size and shape. Not only do the derived elements, particularly those destined to become part of the secondary xylem, undergo expansion in length and more especially in radial dimensions, but the cambial cells themselves alter in size. Many elongate, extension of the tips proceeding slowly in some, spasmodically and at times rapidly in others. Simultaneously other fusiform initials become smaller. Certain aspects of the latter phenomenon were touched upon in an earlier account (Bannan (2)). The results of further investigation are given in the present paper.

Material and Methods

In the present study white cedar or arbor vitae *(Thuja occidentalis* L.) alone was used, and all collections were made within a 50-mile radius of Toronto. Samples including both xylem and phloem were taken at various times during the growing season from the stems of trees of moderate size, mostly 6-12 in. D.B.H. Serial radial, tangential, and transverse sections were cut through the cambium and adjoining secondary tissues from fresh, fixed, or embedded material. Examination of the secondary xylem and phloem provided information on past changes in the cambium, and study of the meristem itself furnished data on current happenings.

In order properly to interpret the alterations in the cambial area it is necessary to determine the extent and sequence of periclinal divisions in that meristem. There must be recognition of the fact that in addition to the initiating layer, which is apparently one cell wide and which determines the basic cell pattern in both xylem and phloem (the cambium as defined

[1] *Manuscript received September 26, 1952.*

Contribution from the Department of Botany, University of Toronto, Toronto, Ontario. Assistance was provided by a grant in aid of research furnished by the University of Toronto.

[2] *Assistant Professor in Botany.*

by some authors), there is usually a zone of periclinally dividing cells on the xylemward side (Fig. 28). The width of this zone varies with time of year, location in the tree, and rate of growth. The zone is usually widest in the stems of vigorous trees and extent is maximal early in the growing season. Later it narrows to one or two cells. Lasting changes in cell pattern in xylem and phloem originate in the uniseriate initiating layer. Local modifications may follow in the periclinally dividing derivatives, resulting in minor differences between xylem and phloem. Often it is necessary to distinguish between changes in the initiating layer and later alterations in the periclinally dividing derivatives which maturate into xylem.

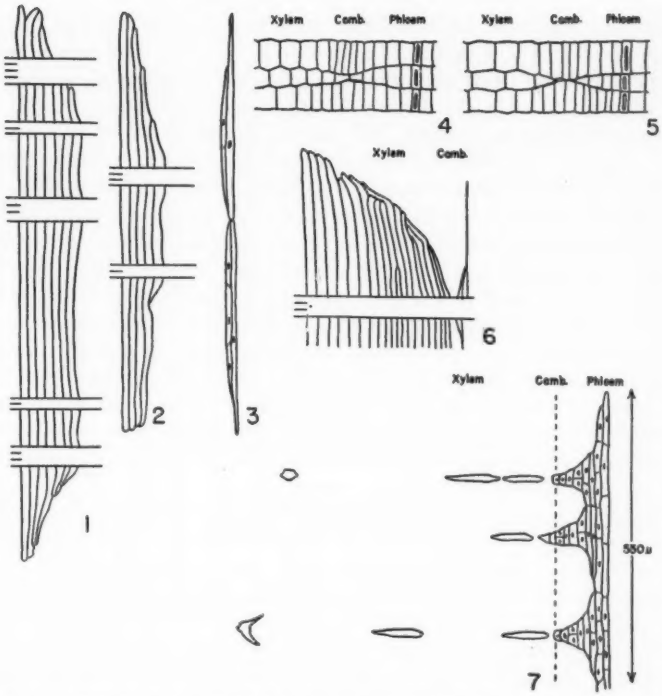
The Reduction of Fusiform Initials

It has been shown in previous publications that increase in girth of the cambium is accompanied by continuous and extensive change in the size, number, and arrangement of the component cells (Bannan (1) and Whalley (6)). The multiplication of fusiform initials, which usually occurs at a relatively rapid rate, is attended with extensive loss, many of the cambial cells undergoing some type of transformation. A few lapse into maturation but the majority are transversely divided, various proportions of the segments shortening to become ray initials. Significantly, this change takes place for the most part in fusiform initials with poor ray contacts (Bannan (3)).

The loss or transformation of fusiform initials is usually accompanied by reduction in cell volume. As a rule this shows up in all planes. In transverse sections the antecedent and penultimate xylem and phloem cells in failing radial files exhibit continuous reduction in tangential width, and the final cell is generally triangular in cross section (Figs. 4 and 5). In serial tangential sections the successive cells seem to shrink until nothing is left. The reduction in tangential dimensions is accompanied by shortening of the cell tips. Amount of the latter ranges from slight to extensive (Figs. 1 and 2).

It would appear that unsatisfactory water relationships are involved in the shrinkage which accompanies loss or transformation of fusiform initials. In competition with their neighbors the failing initials and their immediate derivatives seem unable to acquire or retain water sufficient for normal cell expansion. During the final periclinal divisions increase in cell volume fails to keep pace with cell division. The lag is reflected in reduced tangential and radial dimensions. As the tangentially flattened cells expand radially during periclinal divisions, the new-formed tangential walls are necessarily narrower than in the preceding cells.

Loss in length is in part related to the above circumstances, but directly is the result of asymmetric longitudinal division. Sometimes the tangential wall newly formed in periclinal division slices off to one side short of the cell tip so that the resulting daughter cells are of unequal lengths. Such divisions have been observed both in the initiating row and in the zone of periclinal division on the xylemward side. A frequent concomitant of such asymmetric



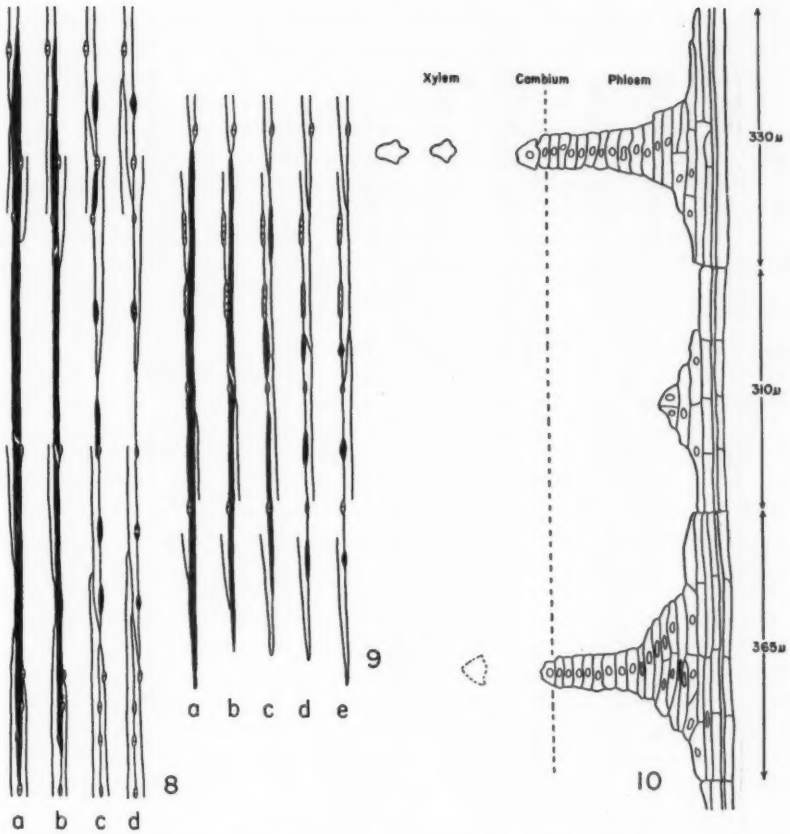
FIGS. 1-7. Drawings from radial or transverse sections showing succession of cells in selected radial rows of secondary xylem or phloem elements. FIGS. 1 and 2. Cessation of radial files of tracheids in secondary xylem, illustrating shortening of the cells. FIG. 3. Termination of file of phloem elements, showing final segmentation. FIGS. 4 and 5. Cessation of radial row of elements in the cambial area, illustrating decrease in tangential dimensions of cells in the failing row. FIG. 6. Stoppage of radial row of cells in xylem, showing reduction in length of the successive cells. FIG. 7. Radial of xylem and phloem showing conversion of fusiform initial into ray initials, and diverse aspects of the xylem and phloem portions of the newly formed rays.

division is reduction in transverse dimensions of the terminal portions of the cells involved. Often the tips of the dividing cells fail to expand radially as much as the central parts, resulting in the development of a somewhat elongate spindle shape. Narrow, lagging tips are a noteworthy feature of the cells in many dying rows. This alteration in cell form doubtless increases the probability of asymmetry in periclinal division. Progressive shortening, such as is illustrated in Fig. 6, is probably the result of a few, i.e., two-four, successive asymmetric divisions in the initiating row of the cambium, the shorter cell functioning as the cambial initial in each instance. The minor irregularities in length among the successive cells shown in the figure are no doubt to be attributed to asymmetry in the later periclinal divisions leading to local multiplication of the xylem derivatives, and to variation in the amount of elongation of individual cells prior to maturation.

Sometimes fusiform initials lapse into maturation relatively abruptly without much loss in length. More often the process is of longer duration with the successive fusiform elements exhibiting progressive shortening at the tips (Fig. 6). Spatial extent of the transitional zone in the xylem marking the period of cambial cell shortening varies to some extent with time of year. If the loss of a fusiform cambial cell occurs during the early part of the growing season, when there is usually rapid and extensive production of xylem elements due to the broad area of local multiplication on the xylemward side of the initiating row in the cambium, amount of xylem produced is usually considerable and the transitional zone may extend across several cells. Toward the end of the growing season, when the zone of periclinal division becomes narrowed to the initiating row in the cambium and perhaps the adjoining row of derived cells on the xylem side, the production of wood is greatly decreased, and the spatial extent of the transitional zone in the xylem may be correspondingly reduced. Quite apart from these seasonal relationships, however, much variation occurs in individual instances in the extent of the transitional zone in the xylem, and the duration and amount of cambial cell shortening.

The final tracheids in radial files which are coming to an end are often more or less abnormal in aspect. They frequently have somewhat narrowed tips, the walls are sometimes thinner than usual, the radial intertracheary pits may be small and irregular in distribution, and sporadic pits appear in the tangential walls even in early wood. In the phloem, deviation occurs from the normal sequence of cell types. Instead of the usual arrangement in which the sequence is sieve cell, parenchyma, sieve cell, and fiber, the final cells generally have persistent nuclei and are parenchymatous in aspect. As a rule the xylem and phloem elements in the dying row do not conform precisely with regard to cell length or occurrence of cross walls. Evidence of segmentation often appears in the phloem (Fig. 3) when none is to be seen in the xylem. In such cases it would seem that the fusiform cambial cell in the initiating row underwent subdivision before the final periclinal division and maturation into phloem parenchyma, while the earlier formed periclinally dividing derivative cells toward the xylem matured to tracheids. The final divisions in the development of the latter cells are frequently asymmetric.

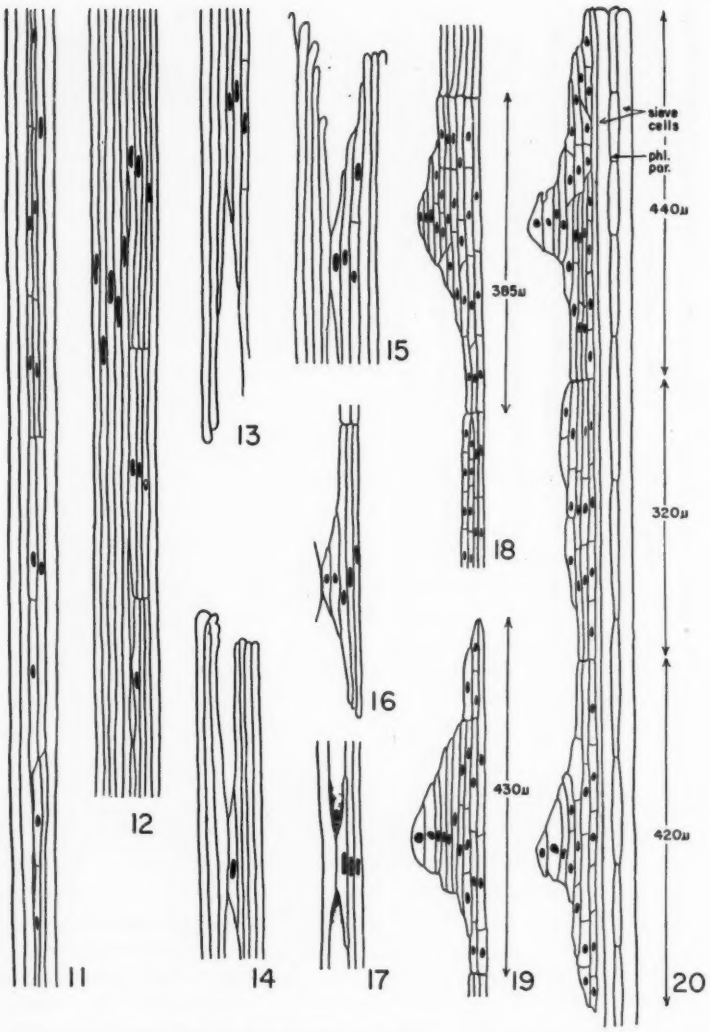
In most cases cambial cells which are about to cease the production of fusiform elements undergo segmentation, after which some of the segments are lost by maturation and others shorten through successive periclinal divisions to become ray initials. Sometimes shrinkage is apparent in the fusiform elements produced immediately before segmentation, while in other instances size reduction begins with subdivision. In any case the former aggressive tendency for elongation is lost. Stages in the segmentation may be ascertained by study of serial tangential sections. Two sequences are depicted in Figs. 8 and 9. It is clear from the succession of phloem elements shown in Fig. 8 that the fusiform initial involved underwent a transverse division (Fig. 8*a*) which was followed by a similar division of the



FIGS. 8-10. FIGS. 8 and 9. Diagrams from serial tangential sections of secondary phloem depicting succession of cells in selected series. The diagrams are to be interpreted as showing stages in the subdivision of fusiform initials and shortening of some of the resulting segments into ray initials. FIG. 10. Radial of xylem and phloem showing conversion of portion of fusiform cambial cell to ray initials.

upper segment (Fig. 8*b*). Thereafter each of the three segments was in turn subdivided to yield a total of six initials which promptly became shorter (Fig. 8*c*). Four of these survived to become ray initials and to initiate new rays (Fig. 8*d*). Fig. 9 illustrates a subdivision to the second order, yielding four shorter cambial units of which three survived to become ray initials.

The number and length of the segments formed by subdivision of fusiform initials vary greatly in different instances, but certain general trends are evident. In the cambium of small branches or stems, where the fusiform initials are relatively short, the number of segments tends to be small, sub-



FIGS. 11-20. Drawings from radial sections of the cambial area showing stages in the subdivision of fusiform initials and conversion to ray initials. FIGS. 11-12. Early stages of subdivision in the cambium. FIGS. 13-17. Asymmetric periclinal divisions in the cambium resulting in shortening of cambial cell segments. FIGS. 18-20. Later stages showing reduction of segments to stature of ray initials.

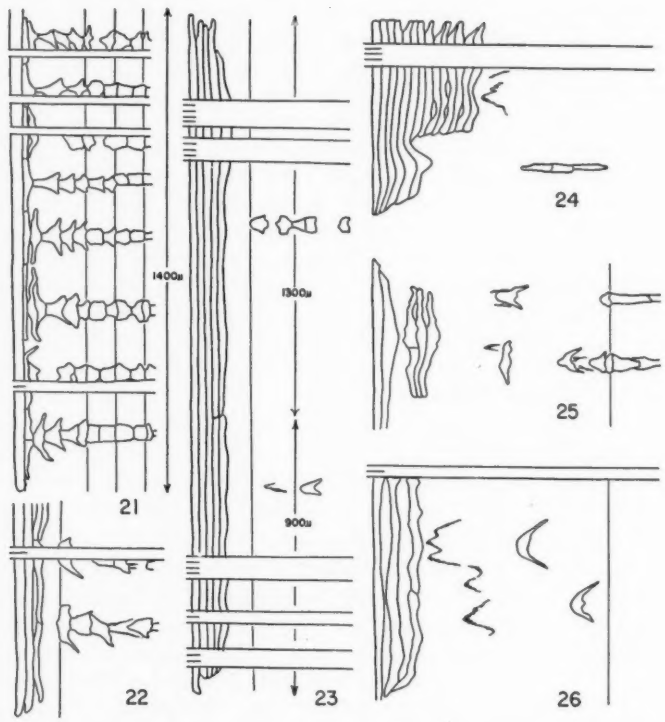
division occurring only to the first or second order yielding two-four parts. In roots the initials are long and subdivision frequently occurs to the third order or beyond to produce several short segments (Fig. 21). In the stems of mature trees the fusiform initials are likewise comparatively long, but the segments are usually longer than in roots. In the particular stems studied average length of the fusiform initials was approximately 2400μ, and for the most part original length of the segments formed by subdivision was in the vicinity of 300-400 μ (Figs. 10, 18, 19, and 20). These data suggest a prevailing tendency for subdivision to occur to the third order, yielding eight segments. However, much variation exists and occasionally subdivision stops with the first transverse division (Fig. 23).

The radial aspect of the earlier phases of segmentation of fusiform initials is shown in Figs. 11-17. These drawings depict the cellular configurations in the cambium, and in Fig. 11 especially, the first two stages in subdivision are to be seen. During the early periclinal divisions of the segments some retardation may occur in the rate of division, accompanied by a slackening in volume increase. Often the upper and lower ends of the cells fail to expand radially as much as the central parts, with the consequence that the tips separate and pull apart. Early stages in this separation are shown in Figs. 27 and 28. Stretching of the plastic pectic substances of the middle lamella across the widening space between the separating cells is apparent. As radial expansion continues in the central parts of the cells but lags toward the tips, the cells become somewhat tapered, spindle-shaped, or elongate plano-convex in outline (Figs. 13-17). When these cells divide periclinally the new-formed walls intersect the older tangential walls short of the cell tips, resulting in daughter cells of dissimilar lengths. The orientation of the cells is such that the shorter cells produced by these asymmetric periclinal divisions remain in the cambium, and the longer cells are added to the xylem or phloem (Figs. 13-17). Through a succession of such divisions the segments become much shortened (Figs. 18-20).

During the period of segmental shortening some retardation usually occurs in the rate of periclinal division, but the degree of lag varies greatly in different instances and from one part of the tree to another. There is also much variation in the size, shape, and type of cell produced. In the cambium of small roots, branches, and stems periclinal divisions typically take place with such frequency as generally to produce radially contiguous cells in both xylem and phloem. In Fig. 21, which depicts the radial aspect in the xylem, it will be noted that most of the cells formed after subdivision are in radial contact. Fig. 22 illustrates the condition in a slightly older specimen where spaces between the cells are more extensive and more common. With increasing cambial age a slowing up occurs in the frequency of periclinal division subsequent to subdivision. Division is usually of such nature and rate as to produce radially contiguous cells in the phloem but only sporadic cells in the xylem. Condition in the phloem remains similar to that in young specimens. Cells formed after subdivision are in radial contact, the successive elements

exhibiting the stages in continuous shortening which arises as a consequence of asymmetric periclinal divisions (Figs. 7, 10, 18-20). As the cells differentiate into phloem elements some remain undivided or undergo a single transverse division (Fig. 30) while in other cases several transverse divisions take place (Fig. 31). Aspect of the corresponding parts in the xylem is highly varied. Occasionally periclinal divisions are frequent enough to produce contiguous cells (Figs. 24 and 29), but more often there are spaces of considerable extent (Figs. 7, 25, and 26). Sometimes gradation occurs from vertically elongate to short and radially extended cells (Fig. 25), whereas in other cases the change from fusiform elements to short ray cells is abrupt (Fig. 23).

The radial files of phloem cells marking the transition from elongate to short elements were noted in a number of conifers by Chrysler (5) who recognized that such radial plates represented a passing phase in the origin



FIGS. 21-26. Drawings from radial sections of the secondary xylem illustrating varied aspects of the first-formed portions of new rays. FIG. 21. Ray origins near center of root showing radial continuity of cells in newly-formed rays. Fig. 22. New rays in slightly older root showing separation of cells. FIGS. 23-26. Ray origins in old stems showing diversity in distribution and shape of cells.

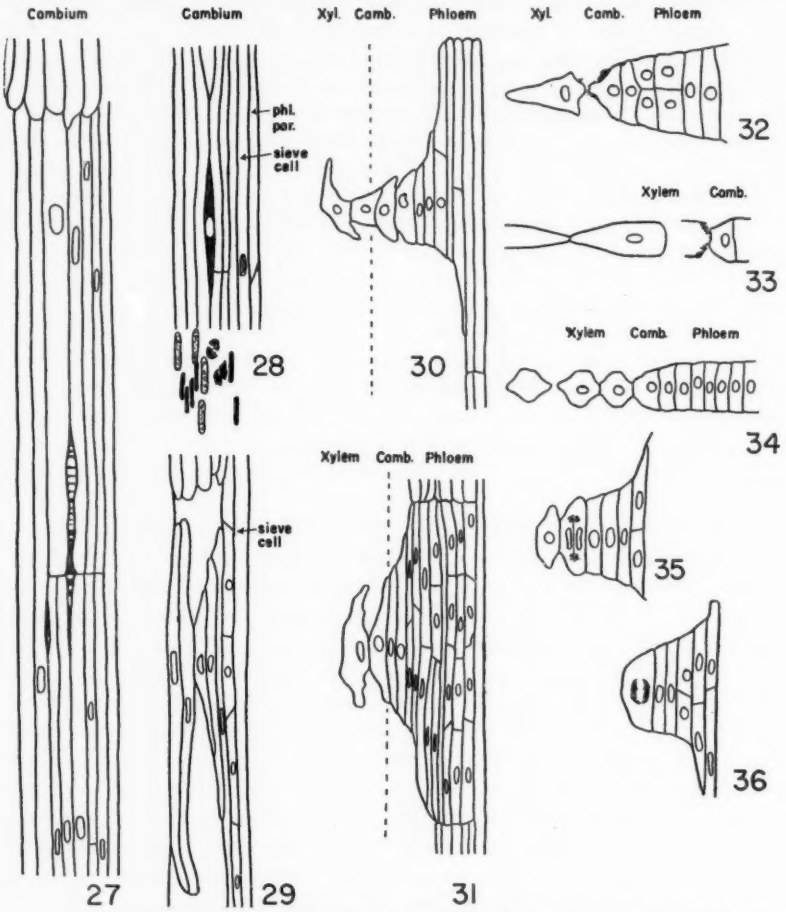
of rays. Barghoorn (4) later described various modes of ray origin, but suggested that the majority of new rays were formed by initials which originated by anticlinal division at the apex or side of fusiform initials. At least in *Thuja* it is clear that the initials of most new rays arise by subdivision of fusiform initials and subsequent shortening of the derived segments.

As the segments of subdivided fusiform initials shorten by asymmetric periclinal divisions some lose their generative capacity and slip into maturation, whereas others continue to shorten until conversion into ray initials is achieved. For example, in Figs. 10 and 20 the median of three nascent ray initials has failed, the upper and lower have successfully undergone the conversion. The actual yield of ray initials from subdivided fusiform initials fluctuates widely both in original production and ultimate survival. On the whole the proportion of failures tends to rise with increasing cambial age.

In the later stages of shortening of the nascent ray initials asymmetric periclinal division and change in shape of the plastic cells are both involved. When production of cells toward the xylem is sporadic the evolving ray initials tend to be more or less plano-convex in outline when viewed radially (Figs. 35 and 36). If radial continuity exists in the developing rays, the cambial cells usually have sloping upper and lower walls which converge toward the xylem. In either case periclinal division results in daughter cells of dissimilar lengths. Usually, but not always, the shorter cell is the next to divide periclinally.

If the nascent ray initials continue producing only sporadic xylem ray cells during the later stages of shortening, these tend to be pulled out in the radial direction (Fig. 32 and 33). Increase in length of the cells takes place partly at the expense of height and the cells become much longer radially and noticeably shorter than the originating cambial cells (Fig. 32). Often, when periclinal divisions toward the xylem are intermittent, the ray cells are pulled apart. Sliding movements occur between the xylem ray cells and the radially continuous fusiform elements on either side. Accumulations of pectic materials of the middle lamella gather which mark former cell positions and contacts later lost (Fig. 33). When periclinal divisions are more frequent the xylem ray cells become more or less diamond shaped (Fig. 34). In such cases the middle lamella seems to have differential adhesive qualities or the wall structure varies such that the median parts of the tangential walls retain contact while the upper and lower parts separate (Figs. 32 and 34). The tendency for radial extension of the xylem ray cells increases as the rate of periclinal division rises and the spaces between consecutive cells diminish. The gradual building up of pulling forces in the radial direction, associated with relative acceleration of periclinal division, acting together with a high degree of wall plasticity and apparent differential adhesive qualities of the middle lamella, doubtless contribute to continued shortening of the evolving ray initials.

The nascent ray initials are also subjected to considerable molding by the adjoining fusiform initials. After subdivision of a fusiform initial the neighboring cambial cells crowd in between the shortening and separating segments, flattening the latter to spindlelike shapes as seen tangentially (Figs. 8 and 9).



FIGS. 27-36. Drawings from radial sections of the cambial area showing stages in the subdivision of fusiform initials and reduction of the segments to ray initials. FIG. 27. Portions of subdivided fusiform initials in the cambium showing separation of the tips of new-formed segments. FIG. 28. Widening intercellular space in cambium located between subdivided cells in initiating layer and periclinally dividing xylemward derivatives of earlier origin. In the lower part of the figure are shown mitoses in laterally adjoining cells, indicating occurrence of periclinal divisions in a zone several cells wide. FIG. 29. Early stages in the shortening of cambial cell segments by asymmetric periclinal division. FIGS. 30-31. Slightly later stages in reduction of cambial cell segments to ray initials, showing differences in size and numbers of transitional phloem cells. FIGS. 32-36. Final stages in the shortening of cambial cell segments to dimensions of ray initials.

Intercellular adjustments in the cambial zone are complex and often circumstances exist or forces are operative which are antagonistic in effect. The expression of cell form varies with the particular conflicting elements of the local environment which are in ascendancy at the critical time of enlargement and differentiation of each cell. The problem of attempting to assess the relative importance and effects of the various forces involved in the determination of cell form is further complicated by the fact that the influence of these forces sometimes varies with the circumstances. The changing relationship between cell shape and rate of periclinal division in the ontogeny of rays may be used as example. Studies indicate that in approximately 40% of all cases, the segmentation of fusiform initials leading to the formation of ray initials takes place during the development of the final quarter of the annual ring. During the deposition of the early wood in the year following ray origin, the production of xylem elements by the shortening cambial cell segments and by the neighboring fusiform initials and their immediate derivatives are usually strikingly dissimilar. Along the axis of the developing ray periclinal divisions are generally limited to the shortening cambial ray cell. Most of the resulting cells are added to the phloem and only sporadic cells accrue to the xylem. By way of contrast, periclinal divisions in the files of fusiform elements on either side occur not only in the initiating cambial cell but also in a xylemward zone several cells wide. Thus, during the early part of the growing season the production of xylem elements by the fusiform initials and their immediate derivatives far surpasses that of the shortening ray initials. The dividing, enlarging, and differentiating fusiform cells tend to expand tangentially so as to occupy the gaps between the new-formed radially separated xylem ray cells. This sealing off of the xylem ray by the adjoining fusiform elements doubtless inhibits xylemward expansion and periclinal division of the nascent ray initials, and limits radial extension of the occasional xylem ray cells that are produced.

During the later stages of ray ontogeny, when the ray initials have shortened, xylemward divisions are usually more frequent and there is greater tendency for the consecutive xylem ray cells to adhere by the median portions of their tangential walls. Sometimes, when divisions do not occur often enough to produce radial continuity among the cells, these become much drawn out horizontally. Accordingly, it would appear that during the earlier phases of ray ontogeny the dissimilar rates of production of ray and fusiform elements in the xylem produce a situation unfavorable to the development and radial extension of xylem ray cells. On the other hand, during the later phases after the ray initials have undergone much shortening, the association of tardiness in periclinal division (albeit not as marked as formerly) and increasing tendency of consecutive cells to adhere by the median portions of their tangential walls facilitate extensive radial elongation.

In the above paragraphs reference has been made to certain local cell relationships which doubtless favor change in shape of the developing ray initials and their products. Such factors, however, probably have only

limited influence and serve merely to modify basic cell form and size determined largely by elements inherent in the cells themselves. One is dealing here with the transformation of one type of meristematic cell to another, i.e., fusiform to ray initial, and as the conversion proceeds alterations take place in important structural and physiological characters. Changes in cell size and form are among the more obvious manifestations of the transformation.

Reduction in height of the nascent ray initials continues until they are only 15-25 μ high. Eventually periclinal divisions occur at such rates and radial extension is of such amount as to produce radial continuity in both xylem and phloem portions of the new vascular rays. During these final stages of ray ontogeny periclinal divisions are not restricted to a uniseriate initiating row in the cambium, but may occur in a zone equally as wide as that concerned with the production of fusiform elements. Occasional transverse divisions follow in the ray initials which, along with the coalescence of recently formed and older rays, result in vertical expansion of the rays.

In conclusion it might be pointed out that the various types of transformation of fusiform initials described above, such as maturation or conversion to ray initials, are not so different as the terms might seem to imply. The early phases are similar. Differences occur chiefly in the duration of the period of change and in the numbers of segments of the subdivided fusiform initials which retain their generative ability. A feature common to nearly all instances of loss or conversion of fusiform initials is early reduction in cell volume, the cambial cells and their immediate derivatives evidently undergoing some physiological change whereby they lose much of their capacity for extension in all directions. This is sometimes followed soon by maturation, segmentation of the fusiform initials frequently occurring just prior to maturation. In the latter case the segmented initial apparently differentiates into phloem cells while the xylemward periclinally dividing derivative cells develop into tracheids. In the majority of cases maturation is in part delayed. The segments of subdivided fusiform initials shorten through successive asymmetric periclinal divisions, some slipping into maturation at various stages of the shortening, others surviving and ultimately becoming reduced to ray initials.

References

1. BANNAN, M. W. The frequency of anticlinal divisions in fusiform cambial cells of *Chamaecyparis*. Am. J. Botany, 37: 511-519. 1950.
2. BANNAN, M. W. The reduction of fusiform cambial cells in *Chamaecyparis* and *Thuja*. Can. J. Botany, 29 : 57-67. 1951.
3. BANNAN, M. W. The annual cycle of size changes in the fusiform cambial cells of *Chamaecyparis* and *Thuja*. Can. J. Botany, 29 : 421-437. 1951.
4. BARGHOORN, E. S., Jr. Origin and development of the uniseriate ray in the Coniferae. Bull. Torrey Botan. Club, 67 : 303-328. 1940.
5. CHRYSLER, M. A. The origin of the erect cells in the phloem of the Abietineae. Botan. Gaz. 56 : 36-50. 1913.
6. WHALLEY, BARBARA E. Increase in girth of the cambium in *Thuja occidentalis* L. Can. J. Research, C, 28 : 331-340. 1950.

THE ISOLATION AND IDENTIFICATION OF SUCROSE FROM THE SEEDLING WHEAT LEAF[1]

By G. M. Ward[2]

Abstract

Crystalline sucrose has been isolated from cleared alcoholic extracts of mature wheat leaves by precipitation as the barium salt. The crystals were positively identified by melting point, X-ray diffraction analysis, and glucose–fructose ratio as well as by conventional analytical techniques. The evidence for the presence of sucrose in the wheat leaf has been corroborated by chromatographic analysis of leaf extracts. It is concluded that sucrose forms the bulk of the soluble sugar reserve of this tissue.

Introduction

The identity of sucrose in the wheat leaf, as revealed by a review of the literature, rests on uncertain evidence. Indeed this is true for many plant tissues, particularly foliage leaves. Most authors have been content to base their claims for sucrose upon analytical procedures which in the absence of some corroborative evidence are known to be unreliable. Belval (5) was one of the earliest authors to report sucrose in wheat leaves on this basis. Newton (15) claimed to have demonstrated the occurrence of sucrose in wheat on the basis of equivalent results from two methods of hydrolysis. Krotkov (13) has reported a correlation between carbon dioxide emission and the disappearance of sugars from the starving wheat leaf and although he did not claim to have identified sucrose, he employed the conventional methods of analysis for its estimation. More recently Bidwell, Krotkov, and Reed (6) have presented chromatographic evidence for the presence of sucrose in wheat leaf tissue. Roberts (18) has reviewed the whole matter of carbohydrates in foliage leaves and has emphasized the uncertainty as to the identity of any of the carbohydrates in wheat leaves. He points out that final and definite proof of the occurrence of sucrose in wheat leaves requires its isolation in crystalline form and the critical proof of its identity. This has been achieved in the research reported in this paper. The evidence is further supported by chromatographic results indicating that sucrose, while undoubtedly present, also represents the bulk if not the whole of the nonreducing carbohydrate of this tissue.

Materials and Methods

The experimental material employed in this investigation was the first foliage leaf of the seedling wheat plant *Triticum dicoccum* Schubler var. *Khapli*. It was grown and prepared for analysis under the standard conditions described by Duff and Forward (10), with the exception of some greenhouse-grown

[1] *Manuscript received September 8, 1952.*

A contribution from the Department of Botany, University of Toronto, Toronto, Ont. An abridgement of part of a thesis submitted in 1952 to the University of Toronto in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] *Chemist, Division of Chemistry, Science Service, Department of Agriculture, Ottawa, Canada.*

material that was used in preliminary tests. The leaves were harvested on the 13th day following a long period of illumination to ensure the maximum content of reserve carbohydrate. They were extracted for four hours with 80% alcohol, the alcohol was removed by vacuum distillation and the water residue was cleared by treatment with a minimum of activated charcoal. The advantage of this latter reagent is that it adds nothing to the solution which cannot be easily removed. The cleared extract was reduced under vacuum to a small volume. A large stock of this material was prepared from successive populations of Khapli wheat, and aliquots were used in subsequent tests. The highly concentrated extract required no preservative but was stored in a refrigerator. After a few days a mass of characteristic potassium nitrate crystals always appeared in these extracts.

For purposes of standardization and measurement it was necessary to adopt one of the conventional analytical procedures. The Hanes modification (11) of the Hagedorn and Jenson ferricyanide method has been used throughout for measuring reducing values. Inversion of sucrose has been carried out by both the acid and the invertase procedures.

The fact that sucrose will form addition compounds with oxides of the alkaline earth metals has been known for many years. It was an application of this reaction which was used in 1883 by Kayser (12) who was the first investigator to claim the isolation of sucrose from leaf tissue. Kayser, working with grape vine leaves, used calcium hydroxide in a method attributed to von Peligot and described by Sachsse in 1877 (19). Darwin and Acton in an old textbook of plant physiology (9) describe a method for the isolation of "cane sugar" from plant extracts using strontium hydroxide as a precipitant for sucrose.

The reaction has found an application in the sugar refining industry where it is used for the partial recovery of sucrose from molasses and low-grade extracts. Here it is known as the Steffen process. Lime is used as the precipitant in all the industrial modifications. The nature of the reaction in the Steffen process has been extensively investigated by French and Belgian sugar chemists (1, 2, 3, 4) who claim that the precipitation of sucrose by calcium oxide is primarily an adsorption phenomenon regulated by the surface extent of the calcium oxide. The compound formed is calcium trisaccharate, but the reaction is not quantitative even with pure solutions. The presence of small amounts of impurities further interferes with the precipitation. The chemistry of the Steffen process has been investigated by Nees (14) who describes a pilot-plant procedure that can be applied with some measure of success on a laboratory scale.

A further modification of the precipitation procedure has been used by Poland (17) in a study of the sugars in the banana. He used the Deguide procedure which employs barium hydroxide as a precipitant for sucrose.

Finally it should be mentioned that sucrose can be precipitated by lead acetate in alkaline medium. An insoluble lead–sugar complex is formed from which sucrose can be recovered by treatment with carbon dioxide as above.

In the course of this investigation all the above-mentioned methods, with the exception of the one involving strontium hydroxide, were applied to wheat leaf extracts. They all have certain features in common although the conditions of temperature, time, and concentration vary considerably. In each case a considerable excess of the particular alkaline earth reagent is allowed to react with a sugar solution. A precipitate of the sugar complex is formed. It is filtered, suspended in water, and treated with carbon dioxide. This releases sucrose from the combination and precipitates the alkaline earth metal as the carbonate. After a further filtration, sucrose remains in solution in the filtrate. The progress of the reaction can be followed by drawing off small aliquots from the original solution and from the two successive filtrates and analyzing them for sucrose by the standard procedure.

In recent years the technique of paper partition chromatography has been applied with considerable success to the separation and identification of sugars and other carbohydrates from biological materials. Since the original work by Partridge was published in 1946 (16) many variations and improvements in the technique have been described in the literature. A wide variety of solvent mixtures and identification sprays is now available for the chromatographic analysis of plant extracts.

In the present investigation one variation of the chromatographic method was applied to wheat leaf extracts to corroborate the evidence of crystal separation for the presence of sucrose in that tissue. A cleared extract was cautiously evaporated to a small volume so that the concentration of sucrose in the final solution was approximately 5%. A spot of this extract was applied to a strip of filter paper and a chromatographic separation was carried out using the butanol – propionic acid – water solvent recommended by Boggs and co-workers (7). Simultaneously a standard sugar solution containing glucose, fructose, and sucrose was chromatographed on the same paper. The sugars were detected and identified with the butanol–aniline – phosphoric acid spray recommended by Bryson and Mitchell (8).

Results

Each of the precipitation methods described above was tested in turn with solutions of pure sucrose and with mixtures of reducing and nonreducing sugars. Under optimum conditions in each case the recovery of sucrose in the final filtrate varied between 75 and 97%. The Steffen process appeared to be a promising procedure and it was tested with crude cane-sugar molasses before being applied to wheat leaf extracts. The recovery of sucrose from molasses was about 25% of the amount present as indicated by the standard inversion procedure. When applied to wheat leaf extracts a recovery of about 55% was obtained. However, the final filtrates were contaminated with traces of calcium which prevented the formation of any crystals even after prolonged standing in the cold or treatment with alcohol. The lead acetate procedure gave a recovery of 70% from a solution of pure sucrose but only 10% recovery from a wheat leaf extract.

The Deguide procedure gave better results than any other method. Recovery of sucrose from solutions of pure sugars varied between 79 and 96%. Recovery of sucrose from wheat leaf extracts varied between 42 and 52%. The final test with the Deguide procedure was made upon an extract of wheat leaves that contained 2220 mgm. of sucrose. Of this quantity 1247 mgm. was precipitated by barium hydroxide and 1150 mgm. was recovered in the final solution.

This final solution was concentrated *in vacuo* to a thick syrup which was treated with absolute ethyl alcohol. A thick sticky precipitate formed which was allowed to stand in the alcohol for several days. A large number of small crystals formed during this time. They were picked out of the sticky solution with a pair of forceps, washed with absolute alcohol, and dried *in vacuo* over phosphorus pentoxide at 78° C. A quantitative recovery of crystals was obviously impossible but a final yield of pure material of 112 mgm. was obtained.

These crystals were critically examined and tested. In shape, appearance, and structure they closely resembled the monoclinic sphenoidal crystals of pure sucrose. A solution of some of the crystals had no reducing power, but after hydrolysis with acid under standard conditions the solution tested 99.0% sucrose. The substance was completely hydrolyzable by invertase. A determination of mixed reducing sugars upon the acid hydrolyzate by the hypoiodite method showed equal quantities of glucose and fructose. The melting point of the crystals was 186.4° C. accompanied by the decomposition and discoloration characteristic of sucrose. This result corresponded to a melting point of pure sucrose determined simultaneously. A determination of mixed melting point produced no change in the result obtained. Finally, some of the crystals were subjected to an X-ray diffraction analysis. The diffraction photograph obtained is reproduced in Fig. 1. This powder pattern is identical with one obtained simultaneously with pure sucrose also shown in Fig. 1 and it conforms in every detail with the X-ray diffraction pattern for pure sucrose described in the A.S.T.M. card index.

A photographic record of the completed chromatogram of a wheat leaf extract is shown in Fig. 2. Column 1 in this figure represents the separation of sugars in the standard. Column 2 shows the sugars in the extract of illuminated wheat leaves. The presence of sucrose is quite apparent. Fructose and glucose, however, cannot be identified in this chromatogram.

The experimental results described here may be accepted as positive evidence of the presence of sucrose in wheat leaf tissue. If due allowance is made for the fact that the precipitation procedures employed in this investigation are not quantitative reactions, and when one considers the small quantities of sucrose involved and the fact that very small amounts of impurities will impede crystal formation, then it may be concluded on the basis of this experimental evidence that sucrose forms the bulk of the non-reducing sugar of wheat leaf extracts and the bulk of the respiratory substrate in the normal, unstarved tissue. The most convincing evidence that this is

PLATE I

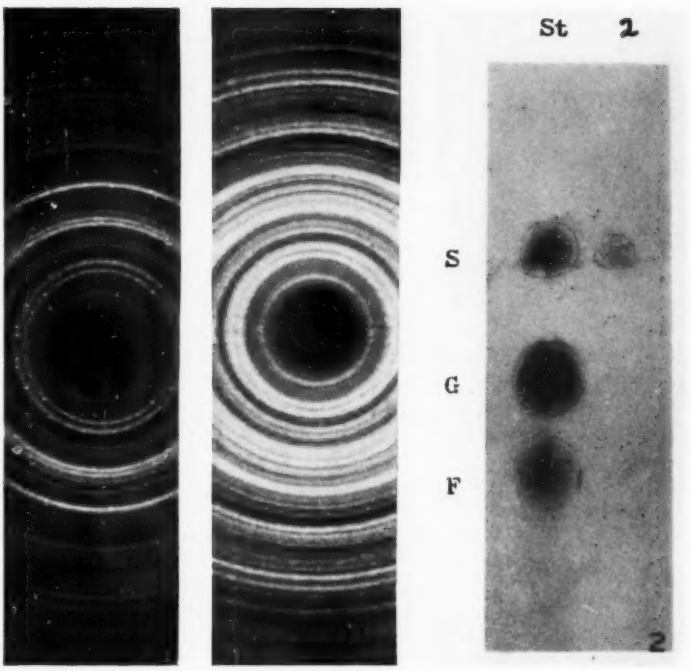


FIG. 1. X-ray diffraction photographs of sucrose crystals.
(*a*) Wheat leaf sucrose. (*b*) Standard sucrose.

FIG. 2. Chromatogram of the sugars in a wheat leaf extract.

so comes, however, from the chromatographic results. The fact that the spot for sucrose is the darkest and indeed the only identifiable spot on the chromatogram from this extract is clear evidence that sucrose is present in quantities of the same order of concentration as the standard and that any other carbohydrate that might be present could be there only in negligible quantities.

Roberts (18) has reviewed the evidence based on analytical procedures for the presence of sucrose in the wheat leaf. Much of this work was confirmed in the present investigation, particularly the hydrolysis–time curve, the invertase reaction, the fructose determinations, and a number of specific color tests. While the evidence for sucrose taken as a whole does not rule out the possibility of the presence of another carbohydrate in the sum total of the soluble reserve carbohydrate of the wheat leaf, yet it does indicate that such a substance, if present at all, would exist in relatively small proportion.

Acknowledgment

The writer is deeply indebted to Dr. G. H. Duff of the Department of Botany, University of Toronto, for his constant interest and encouragement throughout this research, also to Dr. D. F. Forward and other members of the staff of the Department of Botany for assistance in the preparation of samples and the performance of routine analysis. Acknowledgment is also due to Dr. W. H. Barnes of the National Research Council of Canada for making the X-ray diffraction analysis of sucrose crystals.

References

1. ANGELESCU, E. and NICOLESCU, I. Calcium saccharates. Bull. assoc. chim. 52 : 374. 1935.
2. ANGELESCU, E. and NICOLESCU, I. Kinetic studies on the desugarization of sugar solutions with lime. Bull. assoc. chim. 54 : 910-925. 1937.
3. ANGELESCU, E., NICOLESCU, I., and TIGOIU, E. Kinetic studies on the desugarization of sugar solutions with lime. 2. Effect of temperature. Bull. assoc. chim. 59 : 13-27. 1942.
4. ANTONESCU, E. and NICOLESCU, I. Calcium saccharates. Z. Ver. Deut. Zucker-Ind. 81 : 700. 1931.
5. BELVAL, A. M. La genèse de l'amidon dans les céréales. Rev. gén. botan. 36 : 308-324, 337-356. 1924.
6. BIDWELL, R. G. S., KROTKOV, G., and REED, G. B. Paper chromatography of sugars in plants. Can. J. Botany, 30 : 291-305. 1952.
7. BOGGS, L., CUENDET, L. S., EHRENTHAL, I., KOCH, R., and SMITH, F. Separation and identification of sugars using paper chromatography. Nature, 166 : 520-521. 1950.
8. BRYSON, J. L. and MITCHELL, T. J. Improved spraying reagents for the detection of sugars on paper chromatograms. Nature, 167 : 864. 1951.
9. DARWIN, F. and ACTON, E. H. Physiology of plants. 1895. p. 293.
10. DUFF, G. H. and FORWARD, D. F. Physiological and biochemical studies in plant metabolism. I. The respiration of the seedling wheat leaf in starvation and ontogeny. Can. J. Research, C, 27 : 125-145. 1949.
11. HANES, C. S. An application of the method of Hagedorn and Jensen to the determination of larger quantities of sugars. Biochem. J. 32 : 99-106. 1929.
12. KAYSER, R. Studies with sucrose in various plants. Landw. Ver. Sta. 29 : 461. 1883.
13. KROTKOV, G. Carbohydrates and respiratory metabolism in the isolated starving leaf of wheat. Plant Physiol. 14 : 203-226. 1939.

14. NEES, A. R. The chemistry of the Steffen process. Ind. Eng. Chem. 30 : 1323-1326. 1938.
15. NEWTON, R. A. Comparative study of winter wheat varieties with special reference to winter killing. J. Agr. Sci. 12 : 1-19. 1922.
16. PARTRIDGE, S. M. Application of paper partition chromatography to the qualitative analysis of reducing sugars. Nature, 158 : 270. 1946.
17. POLAND, G. L., VON LOESECKE, H., BRENNER, M. W., MANION, J. T., and HARRIS, P. L. Identity of sugars in the ripe banana. Food Research, 2 : 403-409. 1937.
18. ROBERTS, D. W. A. Qualitative and quantitative determination of the wheat leaf carbohydrates. Can. J. Research, C, 28 : 754-779. 1950.
19. SACHSSE, R. Die Chimie und Physiologie der Farbstoffe, Kohlenhydrate, und Proteinsubstanzen. 1877.

PHYSIOLOGICAL AND BIOCHEMICAL STUDIES IN PLANT METABOLISM

VII. THE INVERTASE OF THE SEEDLING WHEAT LEAF[1]

By G. M. Ward[2]

Abstract

The press juice from mature seedling wheat leaves possesses a high thermolabile activity in the inversion of sucrose. It is concluded that an invertase is present. The active material is not associated with the coarse particles of the protoplasm but is soluble in the sap and in that condition is very stable in the cold. It is, however, sensitive to drying and precipitation. Some kinetic characteristics of this enzyme resemble those of invertases from other sources. But the Michaelis constant is small by comparison with that of yeast invertase and therefore a relatively high affinity of the leaf enzyme for its substrate is indicated.

Introduction

The hydrolytic enzyme of sucrose is one of the earliest studied and best known of the enzymes. It has been known for many years and has been the subject of numerous research investigations. It is called variously invertase, sucrase, saccharase, and β-h-fructosidase, the last because it attacks sugars which contain the β-h-fructoside residue, i.e. fructose with the β configuration and the 2-4 furanose ring. It is therefore capable of hydrolyzing such sugars as sucrose, raffinose, gentianose, stachyose, verbascose, and hesperonal (12).

Invertase appears to have a very wide distribution in plants and microorganisms. The occurrence and activity of this so-called ubiquitous enzyme have been described for such widely different groups and members of the plant kingdom as *Aspergillus*, *Penicillium*, *Saccharomyces*, *Impatiens nolitangere*, *Convallaria majalis*, *Platanus orientalis*, *Solanum indicum*, sugar cane, sugar beet, corn, rye, and many others (1, 3, 4, 13, 16). Almost all the organs of the higher plants have been found to possess invertase activity. The effect of various external factors upon the magnitude of enzymic activity has been studied. For example, potassium starvation will cause abnormally high values in sugar cane (7). The same is true for rye sprouts (5), while variations in phosphorus and nitrogen content of the soil appear to have little or no effect upon invertase (5).

The highest concentrations of invertase activity that have yet been encountered occur in various strains of yeast. Yeast is the common source of almost all commercial preparations, and it is the yeast invertase that has been studied most extensively and that has been used in studying the properties of the

[1] *Manuscript received September 8, 1952.*

A contribution from the Department of Botany, University of Toronto. An abridgement of part of a thesis submitted in 1952 to the University of Toronto in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] *Chemist, Division of Chemistry, Science Service, Department of Agriculture, Ottawa, Canada.*

14. NEES, A. R. The chemistry of the Steffen process. Ind. Eng. Chem. 30 : 1323-1326. 1938.

15. NEWTON, R. A. Comparative study of winter wheat varieties with special reference to winter killing. J. Agr. Sci. 12 : 1-19. 1922.

16. PARTRIDGE, S. M. Application of paper partition chromatography to the qualitative analysis of reducing sugars. Nature, 158 : 270. 1946.

17. POLAND, G. L., VON LOESECKE, H., BRENNER, M. W., MANION, J. T., and HARRIS, P. L. Identity of sugars in the ripe banana. Food Research, 2 : 403-409. 1937.

18. ROBERTS, D. W. A. Qualitative and quantitative determination of the wheat leaf carbohydrates. Can. J. Research, C, 28 : 754-779. 1950.

19. SACHSSE, R. Die Chimie und Physiologie der Farbstoffe, Kohlenhydrate, und Proteinsubstanzen. 1877.

PHYSIOLOGICAL AND BIOCHEMICAL STUDIES IN PLANT METABOLISM

VII. THE INVERTASE OF THE SEEDLING WHEAT LEAF[1]

By G. M. Ward[2]

Abstract

The press juice from mature seedling wheat leaves possesses a high thermolabile activity in the inversion of sucrose. It is concluded that an invertase is present. The active material is not associated with the coarse particles of the protoplasm but is soluble in the sap and in that condition is very stable in the cold. It is, however, sensitive to drying and precipitation. Some kinetic characteristics of this enzyme resemble those of invertases from other sources. But the Michaelis constant is small by comparison with that of yeast invertase and therefore a relatively high affinity of the leaf enzyme for its substrate is indicated.

Introduction

The hydrolytic enzyme of sucrose is one of the earliest studied and best known of the enzymes. It has been known for many years and has been the subject of numerous research investigations. It is called variously invertase, sucrase, saccharase, and β-h-fructosidase, the last because it attacks sugars which contain the β-h-fructoside residue, i.e. fructose with the β configuration and the 2-4 furanose ring. It is therefore capable of hydrolyzing such sugars as sucrose, raffinose, gentianose, stachyose, verbascose, and hesperonal (12).

Invertase appears to have a very wide distribution in plants and microorganisms. The occurrence and activity of this so-called ubiquitous enzyme have been described for such widely different groups and members of the plant kingdom as *Aspergillus*, *Penicillium*, *Saccharomyces*, *Impatiens nolitangere*, *Convallaria majalis*, *Platanus orientalis*, *Solanum indicum*, sugar cane, sugar beet, corn, rye, and many others (1, 3, 4, 13, 16). Almost all the organs of the higher plants have been found to possess invertase activity. The effect of various external factors upon the magnitude of enzymic activity has been studied. For example, potassium starvation will cause abnormally high values in sugar cane (7). The same is true for rye sprouts (5), while variations in phosphorus and nitrogen content of the soil appear to have little or no effect upon invertase (5).

The highest concentrations of invertase activity that have yet been encountered occur in various strains of yeast. Yeast is the common source of almost all commercial preparations, and it is the yeast invertase that has been studied most extensively and that has been used in studying the properties of the

[1] *Manuscript received September 8, 1952.*

A contribution from the Department of Botany, University of Toronto. An abridgement of part of a thesis submitted in 1952 to the University of Toronto in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] *Chemist, Division of Chemistry, Science Service, Department of Agriculture, Ottawa, Canada.*

enzyme. The now historic investigation by Michaelis and Menten (11) of the effects of substrate concentration upon enzyme activity in general was carried out with yeast invertase.

The invertases of foliage leaves are however less well known even as our knowledge of the occurrence and metabolism of sucrose in leaves is somewhat fragmentary. In a study of the metabolism of sucrose in the first foliage leaf of the wheat plant it has been shown in an earlier report (17) that sucrose is the primary reserve carbohydrate of that tissue. It is the purpose of this paper therefore to examine the invertase of the wheat leaf.

Invertase, like many other enzymes, is soluble in water, and this property is, of course, employed in most of the methods of preparation (10, 2). In the present research several attempts were made to obtain an enzyme preparation by extracting dried wheat leaf tissue with water. Treatment of the resulting extract with acetone in each case produced a copious precipitate, but none of the preparations so obtained were active in respect to the inversion of sucrose. It was subsequently found however, that the press juice obtained by passing fresh green wheat leaves through a hand-operated juice extractor possessed a high enzymic inversion activity. No further attempts were made therefore to isolate the enzyme by water extraction, and the press juice preparation was used exclusively for a study of its properties. By using the standard wheat leaf material grown and treated according to the specifications of Duff and Forward (6) and by further standardizing the conditions of harvesting and preparation it was possible to reproduce repeatedly a juice which had approximately the same range of activity. A study of the kinetics of the enzyme in this preparation was carried out. The data obtained are presented in the following sections.

Preparation and Assay

The press juice employed in these experiments was prepared as mentioned above by passing wheat leaf tissue through the hand-operated juice extractor. Standard 13-day wheat leaves were used and the operation was carried out immediately after harvesting. The yield of juice obtained was usually about 60 – 70% of the weight of the fresh green leaves. A fresh supply of juice was prepared for each experiment described in this paper. In every instance the juice was a thick green suspension which was immediately centrifuged. A good deal of the suspended material was eliminated in this way, but the supernatant solution was still green and opaque. A little toluene was added as a preservative and the solution was stored in a refrigerator at a temperature just above freezing. Within 48 hr. all the remaining suspended green matter had coagulated and settled. The solution was centrifuged again, leaving a clear yellowish or reddish colored liquid which took on a deeper red color upon prolonged standing in the cold. In all subsequent tests of the enzyme this solution was used without further treatment or dilution. The enzymic activity of the solution decreased only gradually over a period of months if toluene was present.

The green fibrous residue from the juice extractor contained about 75% moisture. When this material was resuspended in water and extracted for 24 hr. it was found to yield only a very slight enzymic activity, e.g. 18 mgm. of sucrose inverted per hour compared with 200 mgm. per hour inverted by a corresponding amount of juice. These results would seem to indicate that the wheat leaf invertase is a soluble enzyme and in this respect it resembles other known invertases. It does not appear to be intimately associated or closely bound up with the coarse particulate matter of cells. This conclusion is not supported however by the observations of Sisakyan and Kobyakova (14) who claim that invertase, amylase, and protease were observed in chloroplasts, chromoplasts, and leucoplasts in a more or less stable adsorbed condition and that they go into solution only after autolysis. Their conclusion that plastids can be regarded as the depot for biocatalysts would seem to be somewhat premature.

Following is an account of the standard procedure used for testing invertase activity.

To a 50 ml. volumetric flask were added the following:

20 ml. of buffer solution to maintain a pH of 4.5
(8.08 ml. of 0.2*M* Na_2HPO_4 + 11.92 ml. of 0.1*M* citric acid),

5 ml. of 10% sucrose solution,

5 ml. of enzyme preparation.

The solution was immediately made up to volume and allowed to stand at the selected temperature for the selected length of time. In the first few experiments room temperature was used, but subsequently the digestion mixtures were placed in a constant temperature bath at 27.5° C. At the end of a selected period of time a 5 ml. aliquot was pipetted from the digestion mixture into a few milliliters of lead acetate solution in a centrifuge tube. The sample was cleared, de-leaded with a little solid sodium carbonate, and then tested for reducing power. Simultaneously two blanks were prepared in 50-ml. volumetric flasks with the following solutions:

1. 20 ml. of buffer solution
   5 ml. of 10% sucrose solution;
2. 20 ml. of buffer solution
   5 ml. of enzyme solution.

These blanks were treated and tested in the same manner as the digestion mixture and the sum of the reducing values of the blanks was subtracted from that of the digestion mixture.

In the absence of any standardized method of measuring and expressing invertase activity, the method of initial rates was chosen and a convenient arbitrary scale was selected for use in the present series of tests, i.e. intial rate expressed as milligrams of sucrose inverted in one hour under the conditions described above. This was always a calculated value obtained from a measure-

ment of reducing power taken during a short enough period to ensure that the inversion was still a unimolecular reaction, i.e. the progress curve was a straight line from the origin.

These conditions having been defined, the first experiment was the construction of a number of progress curves in order to determine the limits of activity of the enzyme. These curves were found to be of the conventional type. A representative example has been selected and is presented in Fig. 1.

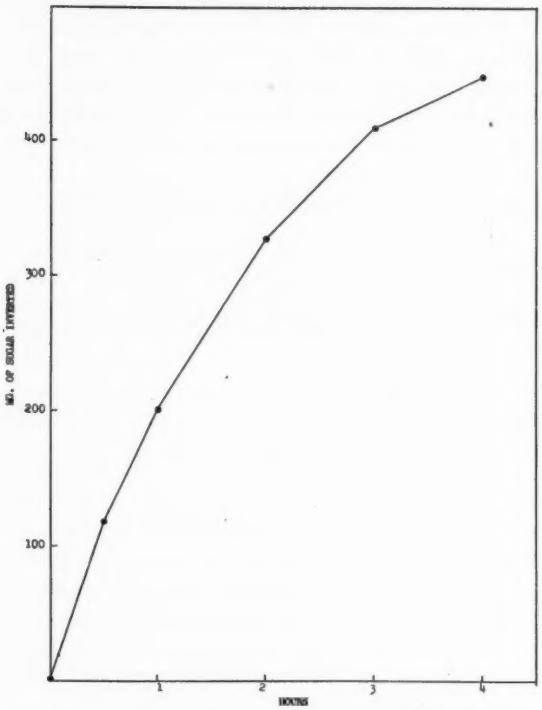


FIG. 1. Typical progress curve indicating enzyme activity.

Stability

The stability of the preparation was then tested by measurement of its activity after various intervals of time. The activity decreased steadily as indicated by the results presented in Table I but the preparation appeared to be sufficiently stable to allow a series of tests to be conducted on any one day without an appreciable change in the performance of the enzyme. In spite of this proof however, the extra precaution was observed, in all subsequent enzyme tests reported here, of starting all digestions in each series at an identical point of time.

TABLE I

ACTIVITY TESTS OF WHEAT LEAF ENZYME SHOWING PROGRESSIVE DECREASE

Date of test	Age of preparation (days)	Inversion activity (mgm. sucrose per hour)
Dec. 17	6	149.1
Dec. 26	11	133.0
Jan. 3	19	103.6
Mar. 25	100	51.3

Optimum pH

In characterizing any enzyme, the pH of optimum activity is an important factor. As previously stated, the enzyme in the wheat leaf preparation responsible for sucrose inversion was found to possess an appreciable activity at a pH of 4.5 which is generally considered to be the optimum for invertase (4, 5, 12). It was decided however, to make a determination of the pH of optimum activity.

A series of digestion mixtures was prepared by appropriate adjustment of the buffer solution with a range of pH values from pH 3 to pH 8. The initial rate of inversion for each mixture was determined. The results are shown graphically in Fig. 2. The experiment was repeated at a later date with another enzyme preparation and although the activity of the second solution was somewhat below that of the first, the results were essentially the same, the two curves were almost identical, and the pH of optimum activity was found to be 4.5 in both cases. This result supports the assumption that the sucrose hydrolytic enzyme in the press juice preparation is invertase.

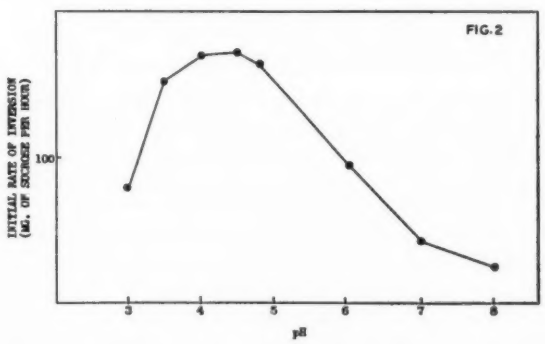


FIG. 2. Effect of pH upon enzyme activity.

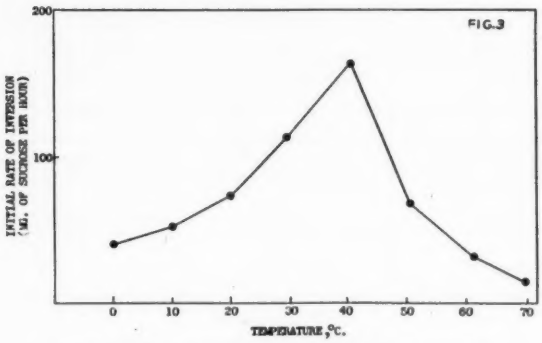


FIG. 3. Effect of temperature upon enzyme activity.

Optimum Temperature

The term "temperature of optimum activity" is sometimes used in the characterization of an enzyme. This term, however, is apt to be misleading unless it is understood that the expressed activity at any given temperature is the resultant of two opposing factors namely (1) increase in the speed of chemical reaction with rising temperature — the well known van t'Hoff relationship and (2) inactivation of the enzyme with rising temperature. The point of balance between the accelerating effect of temperature upon the chemical reaction involved and the destructive effect of temperature upon the enzyme is known as the temperature optimum. This point is somewhat indefinite and varies considerably with the conditions of the reaction.

A determination of the temperature of optimum activity was made with wheat leaf invertase. A series of identical reaction mixtures was prepared and enzymic digestion was allowed to proceed at eight different temperatures regulated at 10° intervals between 0° and 70° C. The initial rate of enzymic inversion was determined for each digest. The results of the experiment are shown in the optimum temperature curve plotted in Fig. 3.

Michaelis' Constant

Many enzyme studies have dealt with various phases of the significance of the Michaelis constant or K_m of the enzyme. The velocity of any enzyme reaction varies with the concentration of substrate present in the digest, and the Michaelis constant may be defined as that substrate concentration which corresponds to one-half the maximum velocity. The derivation of the equation for K_m and all the methods of calculating the constant had their origin in the now classical theory of Michaelis and Menten (11) which postulates the formation of an enzyme – substrate complex in every enzymic reaction before the formation of the ultimate products of reaction.

A brief investigation of this property was carried out with wheat leaf invertase. A series of digests was prepared in which the substrate concentra-

TABLE II

VARIATION OF ENZYME ACTIVITY WITH CONCENTRATION OF SUBSTRATE IN THE DIGEST

[S], gm./50 ml.	[S], molar	1/[S], gm./50 ml.	1/[S], molar	log 1/[S], gm./50 ml.	log 1/[S], molar	V, mgm./hr.	1/V, mgm./hr.	1/V, moles/hr.
0.05	.0029	20.0	342.0	1.301	2.534	80.8	.0124	4233
0.1	.0058	10.0	171.0	1.000	2.233	107.4	.0093	3184
0.2	.0117	5.0	85.5	0.699	1.932	135.8	.0074	2528
0.4	.0234	2.5	42.8	0.398	1.631	158.6	.0063	2156
0.5	.0292	2.0	34.2	0.301	1.534	166.2	.0060	2058
1.0	.0585	1.0	17.1	0.000	1.233	173.8	.0058	1968
3.0	.1755	0.33	5.7	−1.523	0.756	185.4	.0054	1845
5.0	.2924	0.20	3.4	−1.301	0.534	192.2	.0052	1780

NOTE: [S] = *substrate concentration.*
V = *velocity of reaction, initial rate.*

tions were varied from about $0.003M$ to $0.3M$ sucrose. The initial velocity was determined for each digest and the data so obtained were used in the calculations of K_m. In Table II the experimental data are presented. An estimation of K_m was made from these figures by four well known methods (8, 9). Three of these methods are graphical. In the first, the velocity, V, is plotted against substrate concentration, [S], in the second method, V is plotted against log $1/[S]$, and in the third method $1/V$ is plotted against $1/[S]$. The fourth method is an algebraic procedure and the calculation is given herewith.

$$\begin{aligned} .0124 &= 20\ b + a \\ .0074 &= 5\ b + a \\ .0060 &= 2\ b + a \\ .0054 &= .3b + a \end{aligned}$$

$$\begin{aligned} .0312 &= 27.3b + 4a \\ .0266 &= 13.7b + 4a \end{aligned}$$

$$.0046 = 13.6b$$

$$b = .000338 \left(\frac{K_m}{V_{max}}\right)$$

$$\begin{aligned} .0093 &= 10\ b + a \\ .0063 &= 2.5b + a \\ .0058 &= 1\ b + a \\ .0052 &= .2b + a \end{aligned}$$

$$\begin{aligned} .0266 &= 13.7b + 4a \\ .0312 &= 27.3b + 4a \end{aligned}$$

$$.0578 = 41.0b + 8a$$

$$a = .005493 \left(\frac{1}{V_{max}}\right)$$

$$K_m = \frac{b}{a} = \frac{.00338}{.005493} = .0615 \text{ gm. per 50 ml.}$$

By all four methods there was approximate agreement in the determination of K_m, the value being approximately $0.004\ M$ sucrose. The concordance of the evaluation by the several methods gives some evidence that only one enzyme in the unpurified wheat leaf press juice, namely invertase, is responsible for the inversion of sucrose in the prepared digests.

The value for the Michaelis constant obtained in these experiments is considerably lower than those reported in the literature for yeast invertase which

range from 0.017 to 0.040 (12, 15). It has been pointed out that the K_m of invertase varies considerably depending upon the source of the enzyme although the degree of purification appears to have no effect. Various batches of even the same strain of yeast will yield products with decidedly different values for the constant. This fact thus eliminates the possibility of using the Michaelis constant as a means of identifying any enzyme.

Supplementary Enzyme Tests

There can be little doubt that the preparation described in the preceding sections contained the enzyme invertase. The preparation was, of course, not pure and this fact probably caused some interference in the study of the kinetics of the enzyme. A number of additional tests were carried out with this preparation and with various other enzymes available commercially. The significant results may be summarized as follows:

1. Wheat leaf enzyme preparation has no hydrolytic effect on maltose. This result practically eliminates the possibility of the presence of maltose or any maltose-producing carbohydrate in the wheat leaf.

2. Taka-diastase, which contains a small concentration of maltase, has no hydrolytic effect on a wheat leaf extract. This corroborates the above conclusion.

3. Wheat leaf enzyme has only a very slight hydrolytic effect on inulin even after an 18 hr. digestion. This is characteristic of the hydrolysis of certain fructosans.

Acknowledgment

The author is indebted to Dr. G. H. Duff of the Department of Botany, University of Toronto, for his constant interest and encouragement throughout the course of this research, and for valuable suggestions in the preparation of the manuscript.

References

1. ARCHBOLD, H. K. Saccharase activity in the barley plant. Biochem. J. 34 : 749-763. 1940.
2. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis. 5th ed. A.O.A.C. Washington, D.C. 1940.
3. BAMANN, E. and MYRBACK, K. Die Methoden der Ferment forschung. Vol. 2. 1941. pp. 1725-1744.
4. BLAGOVASCHENSKI, A. V. and SOSSIEDOV, N. I. The specific action of plant ferments. 1. The specific conditions of action of leaf invertase. Biochem. J. 19 : 350-354. 1925.
5 DOBY, G. and BODNAR, A. Potassium or phosphate deprivation and the invertase of rye sprouts. Fermentforschung, 14 : 250-255. 1934.
6. DUFF, G. H. and FORWARD, D. F. Physiological and biochemical studies in plant metabolism. 1. The respiration of the seedling wheat leaf in starvation and ontogeny. Can. J. Research, C, 27 : 125-145. 1949.
7. HARTT, C. E. Potassium deficiency in sugar cane. Botan. Gaz. 88 : 229-261. 1929.
8. LINEWEAVER, H. and BURK, D. The determination of enzyme dissociation constants. J. Am. Chem. Soc. 56 : 658-666. 1934.
9. LIPKA, J. Graphic and chemical computations. John Wiley & Sons, Inc., New York. 1921.

10. LUTZ, J. G. and NELSON, J. M. Preparation of highly active yeast invertase. J. Biol. Chem. 107 : 169-177. 1934.
11. MICHAELIS, L. and MENTEN, M. L. Die kinetic der invertinwirkung. Biochem. Z. 49 : 333-369. 1913.
12. NELSON, J. M. Enzymes from the standpoint of the chemistry of invertase. Chem. Revs. 12 : 1-42. 1933.
13. OPENHEIMER, C. Die fermente und ihre wirkungen. Suppl. 1, 202. 1936.
14. SISAKYAN, N. M. and KOBYAKOVA, A. M. The activity and condition of enzymes in plastids. Biokhimiya, 13 : 88-94. 1948.
15. SUMNER, J. B. and MYRBACK, K. The Enzymes. Vol. 1, Part 1. Chemistry and mechanism of action. Academic Press, Inc., New York. 1950.
16. TAUBER, H. and KLEINER, I. S. Some enzymes of *Solanum indicum*. J. Biol. Chem. 105 : 679-682. 1934.
17. WARD, G. M. The isolation and identification of sucrose from the seedling wheat leaf. Can. J. Botany, 31 : 75-80. 1953.

MICROBIOLOGICAL STUDIES OF AIR OVER SOME NONARCTIC REGIONS OF CANADA[1]

By C. D. Kelly and S. M. Pady

Abstract

Using aircraft, studies were carried out on the numbers of bacteria and fungi in the air and 10 separate flights were made during 1948 and 1949 covering territory between Goose Bay, Labrador, and Fairbanks, Alaska. Quantitative samples were taken on agar plates, and silicone coated slides in the McGill G.E. Sampler, as well as in glass wool filters and the results correlated with definite air masses. A comparison of methods showed that the McGill G.E. Sampler gives higher and more consistent results for the total viable count than the filters. Silicone coated slides give the total fungus count including nonviable spores and spores that will not grow on the agar plates.

From various air masses and during different times of the year, there was considerable variation in the average number of bacteria and fungi found. The highest fungus count was 23.6 per cu. ft. and the lowest 0.01. There was less variation in the number of bacteria, the highest being 5.6 and the lowest 0.2 per cubic foot. The highest fungus counts were found in continental polar air during the summer months while for the bacteria the highest counts were found in Pacific polar air during the spring and fall. The bacteria and fungi isolated were all soil types.

Introduction

Studies on the fungi and bacteria found in the air of the Arctic were commenced in the summer of 1947 when N. Polunin exposed Petri plates and vaselined slides by hand from slow moving aircraft in the Arctic and sub-Arctic (5, 6). The results of these first samples were sufficiently interesting to warrant further studies on the microorganisms in the Arctic air. Realizing that such crude qualitative methods as exposing Petri plates and slides by hand would not yield precise information, the authors investigated methods that could be used in aircraft and would sample a definite quantity of air. At this time, the only quantitative methods available for use in aircraft were those designed by Proctor (7) which were not too satisfactory for the studies contemplated. In the development of these sampling methods facilities were placed at our disposal to work with the R.C.A.F. and in designing the samplers the engineering staff of Transport Squadron 426 at Dorval was constantly consulted. Sampling methods and techniques were worked out for use in the R.C.A.F. 'North Star' aircraft (1).

In testing the sampling apparatus, it was found that local test flights at Montreal did not provide sufficient time for testing the methods. The three day scheduled flight of Transport Squadron 426 from Montreal to Whitehorse via Edmonton was found to be satisfactory for testing purposes as it provided adequate time and a variety of weather conditions. Four such flights were carried out between September 1948 and August 1949. In addition six other

[1] *Manuscript received September 19, 1952.*

Contribution from the Bacteriology and Botany Departments, McGill University, Montreal, Canada. The work was supported by a grant from the Defence Research Board. The co-operation of the R.C.A.F., particularly Squadron 426, is gratefully acknowledged. Also we wish to express our appreciation for the invaluable technical assistance in preparing this material, of Sharon Layne, Anna Pawlikowska, and Lucia Kapica.

flights were made: one local flight at Montreal, one to Goose Bay, one to Edmonton, one from Fairbanks to Edmonton, one to Winnipeg, and another from Winnipeg to Montreal. The results of these flights and the numbers of bacteria and fungi are reported here. The kinds of bacteria and fungi will be published separately.

Material and Methods

The methods developed for sampling air-borne microorganisms from aircraft were both used in these studies (1). One, called the McGill G.E. Sampler, utilizes a General Electric Electrostatic Bacterial Air Sampler installed in an aluminum box in which outside air is constantly circulated. With this sampler, agar plates are exposed to obtain viable counts of fungi and bacteria, while silicone coated slides (3) are used to catch viable and nonviable fungus spores. With the second sampler, outside air is passed through a glass wool filter to catch the organisms and later measured by an air meter. For both samplers the outside air is drawn into the aircraft, when in flight, through elbows installed in an escape hatch window and conveyed to the samplers by rubber tubing. The medium used in these studies was a modified Czapek's agar with 0.1% yeast extract and 0.5% glucose. Plates were read at the end of five days' incubation at 25° C. and counts made with the unaided eye. In the silicone slide study, lactophenol with cotton blue was added, then a 22 × 50 mm. cover slip. This area, 1100 sq. mm., was examined under low power using a mechanical stage.

The amount of air sampled depended on an estimated load of organisms. In summer for continental tropic air, the McGill G.E. Sampler was run for two to five minutes and filters 10 to 20 min. In polar air the time of sampling was about twice as long. Under winter conditions both samplers were operated up to an hour. The flights were mostly made at an elevation of 6000 to 10,000 feet and an air speed of 190 to 210 m.p.h.

Lack of agreement in counts by the two methods was noted in many cases. As a rule, the filter counts were somewhat lower than those from the McGill G.E. Sampler, especially in air masses where organisms were numerous. Because of these sometimes conflicting results greater emphasis is placed on the McGill G.E. Sampler counts in this report as the efficiency of the G.E. bacterial air sampler has been established (2).

Air Masses

With the kind assistance of the Canadian Meteorological Service at Dorval Airport, Montreal, and at Rockcliffe Airport, Ottawa, an attempt has been made to determine the history of the air masses encountered. From the navigators log and weather maps, definite fronts have been established as accurately as possible for the time the samples were taken.

The types of air encountered on the flights recorded here were predominantly Pacific polar (pP) or continental polar (cP) in origin. Pacific polar air as a rule moves in over southeastern Alaska and northern British Columbia, down through Alberta and Saskatchewan where it may become mixed with

TABLE I

SUMMARY OF DATA ON NUMBERS OF ORGANISMS IN AIR MASSES SAMPLED IN 10 FLIGHTS DURING 1948 AND 1949

Flight		Air* mass	Average altitude	Temp., °C.	Weather	McGill G.E. Sampler			Filters			Slide McGill G.E.	
						No. samples	Bacteria	Fungi	No. samples	Bacteria	Fungi	No. samples	Fungus spores
1. Montreal Goose Bay return	Sept. 29, 1948	ocT	9600		Clear	5	5.6	2.6	1	1.3	0.2		
		aP	8900		Clear	4	1.1	0.8	2	1.1	0.6		
2. Montreal to Edmonton	Oct. 21, 1948	mcP	6380	0 to −1	Cloudy and clear	10	1.1	0.08	3	1.5	1.9		
		opP	6910	− 2	Clear	4	1.1	0.2	—	—	—	3	155.4
Edmonton Whitehorse return	Oct. 22, 1948	pP	7000 10,000	0 to −5	Clear	21	1.8	0.3	6	0.5	0.5		
Edmonton to Montreal	Oct. 23, 1948	pP	7290	+ 5	Clear	2	2.9	0.3	1	0.5	0.6	1	76.8
		opP_1	7290	− 2	Clear	9	1.8	0.2	4	2.4	0.5	2	45.8
		opP_2	7290	− 2	Clear	3	3.0	0.3	—	—	—	—	—
3. Montreal Whitehorse return	Dec. 27, 28, 29, 1948	opP	8900	−6 to −14	Clear	20	0.2	0.14	—	—	—		
		pP	9000 to 12,000	−2 to −11	Clear	21	0.3	0.15	—	—	—		
4. Montreal to Edmonton	Mar. 7, 1949	ccP	6100	−4 to −12	Clear	4	0.5	0.3	4	0.4	0.2		
		pP	6100	0 to + 3	Low cloud	2	4.0	1.6	2	0.6	0.5		
		cP	6100	− 4	Clear	1	0.03	0.6	1	0.3	0.05		
		pP	6100	−10	Clear	2	3.4	0.7	2	0.7	0.2		
Edmonton Whitehorse return	Mar. 8, 1949	cP	9000 to 12,000	−2 to −10	Clear	8	0.8	0.2	8	0.5	0.1		
Edmonton to Montreal	Mar. 9, 1949	cP	9000	−10 to −18	Clear	6	0.6	0.06	6	0.7	0.1		
		Mixed pP, cP	9000	− 6	Clear	1	4.2	9.9	1	2.9	0.1		

5. Montreal to Edmonton	Mar. 17, 1949	cP	9500 and 6400	−10 to −28	Clear	7	0.4	0.01	7	0.8	0.7		
6. Fairbanks to Edmonton	Mar. 30, 1949	pP	8000 and 10,000	−5 to −14	Clear	5	0.2	0.04	5	0.6	0.2		
7. Local Flight, Montreal	June 21, 1949	cT	5000 and 1700		Cloudy, rain	2	0.5	22.6	2	1.1	13.0	1	180.2
		cP	5000 and 1700		Cloudy and clear	2	0.6	13.0	1	0.8	2.2	1	4.2
8. Montreal to Winnipeg	July 18, 1949	cT	6000	+10	Cloudy, rain	4	0.5	3.0	2	3.0	2.5	1	36.3
		mcT	6500	+ 9	Cloudy, rain	3	0.5	6.2	1	0.4	0.9		
		cP	5760	+ 8	Cloudy	3	0.6	11.8	1	1.4	1.1		
9. Winnipeg to Montreal	July 30, 1949	opP	10,400	0	Clear	8	0.6	1.9	4	0.1	0.3	1	7.4
10. Montreal to Edmonton	Aug. 22, 1949	mcP	8000	+10	Clear to cloudy, rain	10	0.7	3.5	4	0.2	0.3	1	16.3
		cP	7240	+12	Cloudy	5	0.8	16.1	2	2.5	4.9	1	123.8
Edmonton Whitehorse return	Aug. 23, 1949	pP	5780	+ 2	Clear and cloudy	10	0.7	1.9	6	0.2	0.3	2	21.6
Edmonton to Montreal	Aug. 24, 1949	pP	5200	+ 7	Clear	3	0.6	5.5	2	0.6	1.4	1	12.2
		mcP	4400	+17	Clear	5	0.7	13.2	2	3.0	8.4	1	71.3
		cP	4800	+ 8	Clear	6	0.7	1.5	1	0.5	0.5		

* *ocT—old continental tropic air; aP—Atlantic polar air; mcP—modified continental polar air; opP—old Pacific polar; pP—Pacific polar; cP—continental polar.*

other air masses and known as mixed or modified Pacific polar (mpP). Continental polar air, (cP), comes down directly through the Canadian Arctic to eastern Canada or to the central plains where it tends to move east and when mixed with other air masses it becomes modified continental polar (mcP). When an air mass becomes stagnant and remains for some time over an area it is called old Pacific polar (opP) or old continental polar (ocP). Continental tropic air (cT) was encountered at times during some of the summer flights and one set of samples was taken in Atlantic polar air (aP). Continental tropic air moves up through the center of the United States while Atlantic polar air comes in over Labrador.

Flight 1. Montreal to Goose Bay and Return, September 29, 1948

On this flight, the results from the McGill G.E. Sampler showed a distinct difference in the load of microorganisms carried by the two air masses (Table I). Greater numbers of both bacteria and fungi were found in the old continental tropic (ocT) air that had come up from the central United States than the Atlantic polar (aP) air that had come from the Atlantic in over Labrador. This was not clearcut with the filter samples and for the fungi the numbers were higher in the aP air.

Flight 2. Montreal to Whitehorse and Return, October 21, 22, 23, 1948.

Flights to Whitehorse and return were made on three successive days, with a stopover for the nights at Edmonton.

On the first day little difference was noted in the viable counts from the two air masses (Table I). The counts were fairly high for bacteria and low for fungi.

Interesting results were found with the fungi between Winnipeg and Edmonton in the old Pacific polar air mass (opP). Alternate samples were taken with plates and slides in the McGill G.E. Sampler and the four sets of plates gave an average count of 0.2 fungus colonies per cu. ft. (Table I). The first two slides showed counts of 277.4 and 188.5 spores per cu. ft. while the third slide gave essentially the same count as the plates (0.5 per cu. ft.) (Table II). The spores on the first two slides were almost one hundred per cent smut spores that would either not grow on the agar in the five day incubation period or were nonviable. It would seem from these results that the air west of Winnipeg to the center of Saskatchewan was heavily loaded with smut spores though this was not apparent from the agar plates.

The second day's flight, to Whitehorse and back to Edmonton, was through a single air mass of Pacific polar air (pP), and the bacterial counts from the McGill G.E. Sampler were much higher than for the fungi and higher than the counts from the filters while the fungus counts showed little difference from the two methods. From the results of the McGill G.E. Sampler it was observed that north of 58° N. the bacterial counts (2.5 per cu. ft.) were consistently higher than south of that point (1.2 per cu. ft.) (Table III). When

TABLE II

DATA FROM INDIVIDUAL SLIDE AND AGAR PLATE SAMPLES TAKEN ON FLIGHTS BETWEEN WINNIPEG AND EDMONTON WITH MCGILL G.E. SAMPLER, OCTOBER 1948

Date	No.	Method	Air* mass	Exp. time minutes	Total cu. ft.	Organisms per cu. ft.		Weather	Temp., °C.	Wind direction	Altitude
						Bacteria	Fungi				
Oct. 21	14	Plate	opP	10	10	2.2	0.2	Clear	0	315	7700
	15	Slide	"	30	30	—	277.4	"	0	315	7700
	16	Plate	"	10	10	0.8	0.0	"	−2	315	6900
	17	Slide	"	30	30	—	188.5	"	−2	315	6620
	18	Plate	"	12	12	1.1	0.2	"	−2	315	6400
	19	Slide	"	60	60	—	0.5	"	−2	315	6500
	20	Plate	"	10	10	0.4	0.4	"	−2	315	6600
Oct. 23	48	Slide	pP	30	30	—	76.8	Slight haze	+5	250	6700
	49	Plate	"	10	10	2.2	0.2	"	+5	250	7600
	50	Plate	"	10	10	3.6	0.4	"	+5	250	7400
				Front							
	51	Slide	opP	30	30	—	46.5	"	+5	300	7200
	52	Plate	"	10	10	2.6	0.2	"	+4	300	7900
	53	Plate	"	10	10	1.6	0.4	"	+4	300	7100
	54	Slide	"	30	30	—	45.1	"	+2	300	6500

* *opP = old Pacific polar; pP = fresh Pacific polar.*

the aircraft reached 58° N. the altitude was increased from 7000 to 9000 ft. to clear the Stikine Mountains and on the return flight the altitude was reduced from 10,000 to 8000 ft. at the same point.

On Oct. 23, in the three air masses, the average numbers of bacteria were high (1.8 to 3.0 per cu. ft.) and fungi were low (0.2 to 0.3 per cu. ft.) with the viable counts (Table I). Between Edmonton and Winnipeg agar plates and slides exposed alternately in the McGill G.E. Sampler gave similar results in the number of fungi to those obtained on Oct. 21 (Table I). On the slides almost one hundred per cent of the spores were smut spores which appeared to be more widespread than at the time of the previous sampling but the numbers were lower (Table II).

The interesting feature of this three day flight is that it was mostly through pP air which had become modified as it moved eastward. Counts of bacteria were higher than for fungi ranging from 1.1 to 3.0 per cu. ft. with the McGill G.E. Sampler while the fungi on the plates were all remarkably uniform being about 0.2 per cu. ft. These bacterial counts were some of the highest obtained in this study (Fig. 4). The filters gave contradictory readings, being lower in the case of the bacteria and higher for the fungi than the McGill G.E. Sampler.

TABLE III

COMPARISON OF NUMBERS OF ORGANISMS FROM AIR SAMPLES TAKEN NORTH AND SOUTH OF 58° N. BETWEEN EDMONTON AND WHITEHORSE FROM 'NORTH STAR' AIRCRAFT

Date	Position	Wind direction	Flight direction	McGill G.E. plates		Temp., °C.	Altitude
				Bacteria*	Fungi*		
Oct. 22, 1948	South of 58°	240° 250°	North South	1.3 1.05	0.4 0.05	− 5 0	7300 8300
	North of 58°	260° 290°	North South	2.15 3.0	0.4 0.5	− 4 − 8	9000 10,400
Mar. 8, 1949	South of 58°	308° 15°	North South	0.3 0.2	0.05 0.07	−11 −10	7000 8800
	North of 58°	324° 350°	North South	0.7 2.0	0.08 0.18	− 4 − 3	8500 10,000

* *Average counts.*

Flight 3. Montreal to Whitehorse and Return, December 27, 28, 29, 1948

Little difference was found in the counts from the opP air in the east and the pP air in the west. The average counts on the agar plates for the three day flight were 0.25 bacteria and 0.15 fungi per cu. ft. The highest single counts obtained were 1.9 for bacteria and 0.6 fungi per cu. ft., while a number of plates remained sterile even though 20 cu. ft. of air was sampled.

This set of samples was of interest because of the very low counts for both bacteria and fungi and would seem to represent the average number of micro-organisms found under typical winter conditions or what might be indicative of the minimum load for this area.

Flight 4. Montreal to Whitehorse and Return, March 7, 8, 9, 1949

On this sampling flight two sets of inlet and outlet elbows were installed which made possible simultaneous sampling with the McGill G.E. Sampler and the filters. Sampling of the same air thus permitted a more satisfactory comparison of the efficiency of the two samplers. Twenty-four simultaneous samples were taken with each sampling time being about one hour in length. The results from the two samplers showed poor agreement, even though the same air was sampled. The McGill G.E. Sampler indicated high numbers in the pP air, for the bacteria particularly, and low in cP and ocP air, while the filter counts were uniformly low in all three air masses (Tables I, IV, Fig. 1). These results where the numbers from filters are much lower than from the McGill G.E. Sampler, have been found elsewhere (4), particularly where the filters were run for a long time.

Here again, between Edmonton and Whitehorse the bacterial counts with the McGill G.E. Sampler were distinctly higher north of 58° N. than south of that point (Table III).

Flight 5 and 6. Montreal to Edmonton, March 17, and Fairbanks to Edmonton, March 30, 1949

On these two flights winter conditions prevailed, similar to those found in December and counts were low with both sampling methods (Table I).

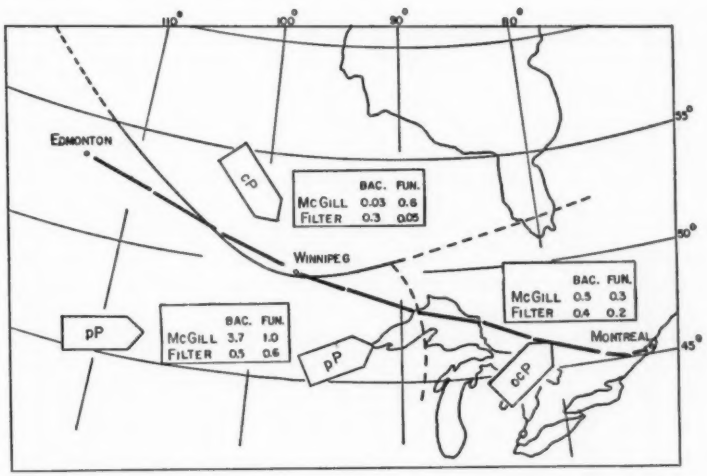


FIG. 1. From Flight 4, Montreal to Edmonton, March 7, 1949. Air masses and wind direction are indicated by solid arrows, with corresponding average counts for bacteria and fungi in a box. Cold fronts are indicated by solid lines and warm fronts by broken lines. The line of flight and individual samples are indicated by heavy lines.

TABLE IV

DATA OF INDIVIDUAL AIR SAMPLES TAKEN BETWEEN MONTREAL AND EDMONTON, MARCH 7, 1949

Sample	Exp. time, min.	McGill G.E. Sampler plates			Filters			Air* mass	Altitude	Wind dir. sp. K	Temp., °C.	Weather
		Total cu. ft. sampled	Organisms per cu. ft.		Total cu. ft. sampled	Organisms per cu.ft.						
			Bacteria	Fungi		Bacteria	Fungi					
1	21	21	0.66	0.76	62.5	0.32	0.16	ocP	2000			Clear
2	60	60	0.73	0.23	100.7	0.72	0.13	"	4000	276 14	−12	"
3	60	60	0.37	0.03	100.0	0.21	0.05	"	5550	248 15	− 8	"
4	60	60	0.4	0.1	120.0	0.29	0.29	"	6000	230 50	− 4	Clear and cloudy
5	60	60	4.16	0.32	120.0	0.76	1.84	pP	6100	236 66	+ 3	Clear
6	60	60	3.9	2.46	103.0	0.38	0.22	"	6700	224 48	0	"
7	60	60	0.3	0.63	89.0	0.34	0.05	cP	6800	317 30	− 4	Clear
8	60	60	2.46	0.13	112.0	1.34	0.13	pP	6500	Nil	−10	Clear
9	60	60	4.41	1.18	118.0	0.07	0.18	"	6100	250 7	−10	"

* *ocP — old continental polar; cP — fresh continental polar; pP — fresh Pacific polar.*

Flight 7. Local Flight near Montreal, June 21, 1949

The data from this flight have been published separately (1) but are included in this series. Samples were taken in two air masses; cP air coming down from the north and cT* air from the south through the eastern United States. This flight indicates the summer conditions with high numbers of fungi particularly in cT air. The numbers of bacteria were quite low.

*Flight 8**. Montreal to Winnipeg, July 18, 1949*

The average bacterial counts from the McGill G.E. Sampler plates in the three air masses were less than one per cu. ft., while the filter gave higher counts in cT and cP air (Table I). The fungus counts were much higher from the McGill G.E. Sampler but about the same as the bacteria from filters. The counts from the cT and mcT air would seem to be low for this type of air, probably owing to precipitation of the organisms by rain. These results show such a complete lack of agreement for both bacteria and fungi with both methods that little can be said about the flight.

Flight 9. Winnipeg to Montreal, July 30, 1949

The average numbers of bacteria and fungi in this series was rather low for this time of year for Pacific polar air that had moved across Canada.

Flight 10. Montreal to Whitehorse and Return, August 22, 23, 24, 1949

On Aug. 22, a cold front was passed at Winnipeg with cP air to the west and an mcP air mass in the east that had moved down from the Arctic into the northern United States and back into Canada. The average number of fungi from agar plates and slides from the McGill G.E. Sampler and from the filters were much lower in the mcP air than samples taken by the same methods in the cP air (Table I). While it might be expected that the mcP air that for some time had been over the wheat growing area of the U.S. and Canada would carry a heavier load of microorganisms than the fresh cP air, the load was probably reduced by rain as thunderstorms were encountered over Lake Superior.

The bacterial counts from the McGill G.E. Sampler showed very little difference in the two air masses (0.7 and 0.8 per cu. ft.) while with the filters the numbers from the cP air were more than 10 times higher than those from the mcP air (0.2 and 2.5 per cu. ft.). This difference may have been due to the fact that with the surface growth on the McGill plates where some of the plates had as many as 157 fungus colonies, the bacteria were inhibited or obscured by overgrowth of the fungus colonies. The colonies in the poured plates from the filters were mostly subsurface and overgrowth by fungi was

** In the previous publication (1) this air had been reported as maritime tropic air, but as it had moved up through the Atlantic States from near Florida, it is reported here as continental tropic.*

*** Data on the flight of July 18, 1949 (Flight 8) as well as those of July 30, 1949 (Flight 9) and Aug. 22, 23, and 24, 1949 (Flight 10) were published by Polunin. Svensk Bot. Tidskr. Bd. 45, H. 2, 1951, Uppsala, without our knowledge or consent. Polunin had no part in this work and the numbers reported for bacteria and fungi appear to be from a preliminary report and are not correct.*

TABLE V

DATA OF INDIVIDUAL AIR SAMPLES TAKEN BETWEEN EDMONTON AND MONTREAL, AUGUST 24, 1949

Sample	McGill G.E. Sampler plates				Filters				Air* mass	Altitude	Wind dir. sp. K	Temp., °C.	Weather
	Exp. time, min.	Total cu. ft. sampled	Organisms per cu. ft.		Exp. time, min.	Total cu. ft. sampled	Organisms per cu. ft.						
			Bacteria	Fungi			Bacteria	Fungi					
1	15	15	0.7	4.8	60	175.7	0.6	0.7	pP	5200	290 25	+ 7	Clear
2	15	15	0.5	5.9					"	5200	290 25	+ 7	"
3**	30	30	1.0	5.9	91	179.0	0.5	2.1	"	5200	290 25	+ 7	"
4	15	15	0.7	21.1					mcP	5300	290 25	+ 7	In clouds
5	15	15	0.8	10.9					"	5300	200 25	+18	"
6	15	15	0.3	4.1	60	128.4	4.0	7.7	"	7000	230 30	+19	Over cloud
7	15	15	0.5	13.9	60	141.1	2.0	9.0	"	6800	230 30	+15	"
8	30	30	0.1	23.2					"	7000	220 30	+15	Clear
9	15	15	0.3	0.3	106	192.8	0.5	0.5	cP	7000	180 25	+12	Clear
10	15	15	0.5	0.3					"	6700	90 20	+10	"
11	30	30	0.3	0.9					"	6700	85 20	+ 9	"
12	15	15	0.0	0.9					"	7000	85 15	+ 9	"
13	15	15	0.7	1.5					"	7000	85 15	+ 9	"
14	15	15	2.3	5.1					"	7000	85 15	+ 9	"

* *pP—fresh Pacific polar; cP—fresh continental polar; mcP—modified continental polar.*
** *This filter was exposed in pP air for 36 min. and in mcP air for 55 min.*

prevented, which reduced the possibility of inhibiting or obscuring the bacterial colonies. Under these conditions, the filters might give a clearer picture of the bacterial load.

The samples taken between Edmonton and Whitehorse, Aug. 23, were all in fresh pP air and were uniformly low. No difference in numbers of bacteria north and south of 58° N. as found in October and March was noted at this time.

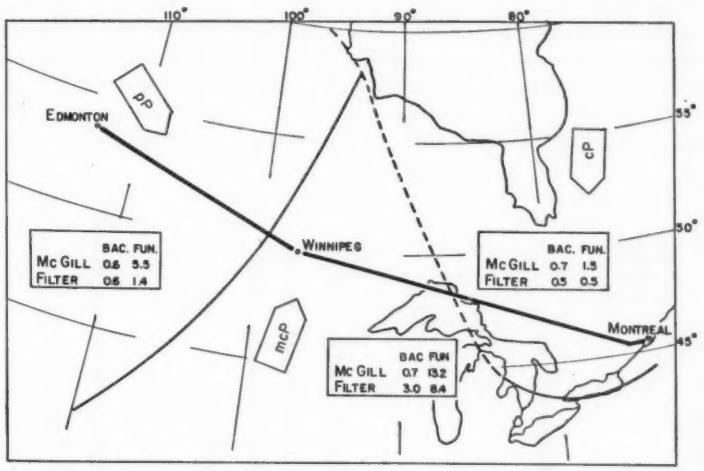


FIG. 2. From Flight 10, Edmonton to Montreal, August 24, 1949. Air masses and wind direction are indicated by solid arrows, with corresponding average counts for bacteria and fungi in a box. Cold fronts are indicated by solid lines and warm fronts by broken lines. The line of flight is indicated by a heavy line.

The flight from Edmonton to Montreal on Aug. 24 was through three air masses that showed some difference in the load of microorganisms (Fig. 2, Table V). The flight from Edmonton was in pP air to a point 160 miles west of Winnipeg, then through mcP air to the eastern edge of Lake Superior and the remainder of the trip was in cP air. On this third day, the fresh pP and cP air carried a noticeably lower number of fungi from both the viable methods and from the slides than the mcP air that had been for some time over the prairies and was mixed with southern air. A difference in numbers of bacteria in these three air masses was noted with the filters, but not with the McGill G.E. plates. Here again where high numbers of fungus colonies were present on the plates as in the mcP air, the bacteria appeared to have been suppressed as the counts from the filters were more than four times higher than from the McGill G.E. Sampler plates.

Air Masses and Numbers of Microorganisms

The most common types of air encountered on these flights for all seasons of the year were Pacific polar (pP), found 13 times and continental polar (cP) air, found 12 times. Continental tropic (cT) air was encountered on five occasions and Atlantic polar (aP) air only once.

An examination of the data was made for variation in the load of microorganisms carried by the various air masses at the time of greatest numbers. As the counts from the filters showed such variation only McGill G.E. Sampler counts are used for viable counts. Particularly over the prairies this showed that higher numbers of fungi were present in cP air than in pP air, while with the bacteria the reverse was noted (Figs. 3, 4). Average counts of fungi in cP air, west of Lake Superior, during July and August were higher (11.8, 16.1, and 13.2 per cu. ft.) than the counts (1.9, 1.9, and 5.5 per cu. ft.) made at the same time in pP air (Fig. 3). Average fungus counts made in cT air at

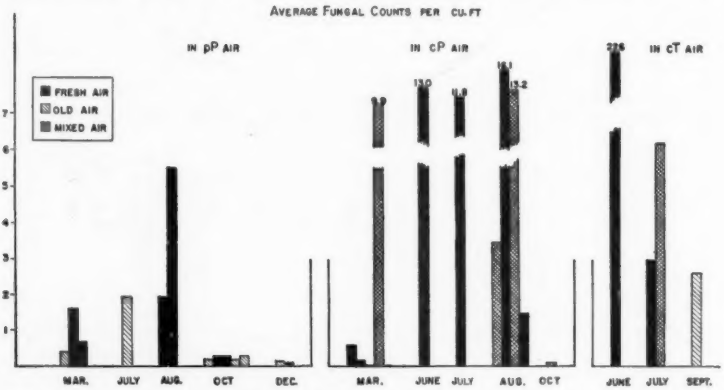


FIG. 3. The average number of fungi found in different air masses and periods of the year. Data are from the McGill G.E. Sampler plates.

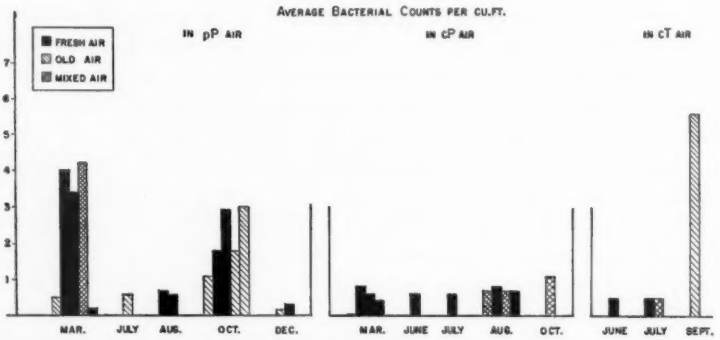


FIG. 4. The average number of bacteria found in different air masses and periods of the year. Data are from the McGill G.E. Sampler plates.

this same time and also in June showed greater variation (23.6, 16.8, 3.0, and 6.3 per cu. ft.). On the other hand, the highest counts for bacteria were in March (4.0, 3.4, and 4.2 per cu. ft.) and October (1.1, 1.8, 2.9, 1.8, and 3.0 per cu. ft.) and were all from pP air. Samples taken in cP air at these same periods showed bacteria to be extremely low (0.03, 0.8, 0.6, and 0.4 per cu. ft.) (Fig. 4).

Samples taken in cT air during the summer had fungus counts from 3.0 per cu. ft. to 23.6, and one series in September had 2.6 per cu. ft. The four series taken in cT air during June and July had bacterial counts less than 0.6, while one in September had 5.6. Some of these bacterial counts may have been low owing to overgrowth of the plates by fungi as indicated in a series in June and one in July by McGill G.E. plates and filters (Table I).

Types of Microorganisms from Air Samples

Bacteria

It was not possible to make bacterial examinations of all the plates in these studies, but, in some cases, all the colonies from certain series of plates were examined. Bacteria were isolated from eight series of samples and all were typical soil forms. The 1173 cultures examined were placed in five main groups on morphological grounds.

SUMMARY OF MORPHOLOGICAL TYPES OF 1173 BACTERIA

Types of bacteria	
Gram-negative rods (*Flavobacterium*, *Achromobacter*, or *Pseudomonas*)	4.9%
Gram-positive pleomorphic rods	23.8
Micrococcus	18.8
Sarcina	4.6
Aerobic sporeformers	37.9

Fungi

The principal types of fungi obtained in culture and from the slides are given in Table VI. While many fungi were isolated from the air, only the common-

TABLE VI

SUMMARY OF TYPES OF FUNGI

Types of fungi	Colonies (%)	Spores (%)
Cladosporium	73.0	32.4
Alternaria	7.0	3.3
Penicillium	2.9	—
Streptomyces	2.9	—
Stemphylium	1.5	—
Aspergillus	0.7	—
Yeasts	0.7	—
Nonsporulating	8.2	—
Other fungi	2.8	6.6
Smuts	—	52.4
Helminthosporium	—	0.3
Rusts	—	0.1
Hyphae	—	4.4

est ones are listed here. On the basis of cultures, *Cladosporium* is the commonest fungus, but on the slides smuts were the most numerous with large numbers being obtained on the flight over the prairies in October 1948. The types of fungi were determined from the examination of 3522 colonies and spores on 16 slides obtained on the 10 trips.

Discussion

It was not possible in this study to arrange sampling flights to cover all periods of the year and the type of air was restricted to that encountered on the various flights. While the data do not cover all the months of the year they give a good indication of the variation in the load of microorganisms across Canada in the different seasons and the relationships of numbers to certain types of air.

During certain seasons, for both fungi and bacteria, the numbers carried in the air were higher than for the remainder of the year. With the fungi there was considerable difference between the highest average count in a single series of samples (23.6) and the lowest average count in another set (0.01). The average counts from samples taken during June, July, and August were generally much higher than for the rest of the year. From 13 sets of samples taken during these three months (Fig. 3) only three had less than 2 fungi per cu. ft., while the remainder carried more than 3 fungi per cu. ft. and six of these sets had average numbers greater than 9 fungi. With the exception of one set taken in cT air in September with 2.6 fungi per cu. ft. and one in March from pP air with 1.6 fungi, the averages of all the samples taken during the remainder of the year were less than 1.0 per cu. ft.

From the counts of the bacteria, the difference between the highest and lowest was not as great as noted for the fungi. The highest average count from one series was 5.6 and the lowest was 0.2 bacteria per cu. ft. However, certain series taken during March and from the end of September through October were higher than any taken during the remainder of the year (Fig. 4). This was particularly true of samples taken in pP air or pP air mixed with cP air over the prairies. Of nine sets of samples taken in March, six taken in cP air or pP air north of Edmonton averaged less than 1.0 bacterium per cu. ft. while the other three taken in pP air or mixed pP and cP air over the prairies gave average counts of 3.4, 4.0, and 4.2 bacteria. All the series of samples taken from the end of September through October had average counts greater than 1.0 bacterium per cu. ft. Two samples out of nine taken at this period had averages about 3.0 and one series in cT air had 5.6 bacteria per cu. ft. The counts of series taken at times of the year other than March, late September, and October were 0.8 bacteria per cu. ft. or less.

It is not entirely clear why the numbers of bacteria in samples taken between Edmonton and Whitehorse during October and March should be greater in the northern portion of the flight, over the mountains, than in the south where there was some farm land. Proctor (7) and Wolfe (8) offer evidence to show that, where there is an absence of turbulence, there is sedimentation of bacteria

in the air with an increase in numbers at lower elevations. This being so, the numbers should be higher in the south at 7000 ft. above sea level than in the north at 9000 ft. The elevation above sea level, however, does not give a true picture as at 9000 ft. above the mountains the aircraft was closer to the earth's surface than at 7000 ft. and the air sampled was from a lower stratum.

The reason for high counts at definite seasons of the year is not entirely clear, but the manner in which these organisms are carried into the air would seem to help explain some of the results. The fungi are carried into the air as spores and during the period of greatest fungus growth, spores could be swept into the air in large numbers. The peak load of fungus spores in June, July, and August is undoubtedly due to the fact that it is the period of most prolific fungus growth.

Bacteria in the soil are found in greatest numbers during spring and fall, at a time when there is little vegetative covering and when much of the soil is being cultivated. These periods would seem to give the best opportunity for wind erosion of the soil and a resulting higher number of bacteria in the air during these seasons.

It is difficult to explain the higher counts of bacteria and fungi in certain air masses and further study, particularly as to the history of the air masses, may indicate the original source of the organisms.

In a previous publication (1), a comparison was made of the efficiency of the two sampling methods. At that time it was felt that the McGill G.E. Sampler gave higher counts and had certain advantages over the filter method. In the present study this was again demonstrated to be true, but more particularly for the fungi than for the bacteria. The counts were usually higher from the McGill G.E. Sampler than the filter for both bacteria and fungi. However, in some cases where large numbers of fungus colonies obscured the bacterial colonies or inhibited their development, the counts from the filters did have an advantage in demonstrating a variation in numbers of bacteria in different air masses as shown in samples taken Aug. 22 and 24, 1949. Where numbers of either bacteria or fungi were greater than 1.0 per cu. ft. the McGill G.E. Sampler always gave higher counts on the filters. On the other hand, counts from the filters were higher only when numbers in the air were low. With low numbers, around 0.05, there was always a chance of greater error from contamination. Because of low counts and erratic results from the filters, most of the discussion in this paper is based on counts from the McGill G.E. Sampler.

The value of silicone slides has been demonstrated in determining the total fungus spore count which includes those that will or will not grow on agar and also nonviable spores. Agar plate samples taken Oct. 21 and 22, 1948, show counts ranging between 0.2 and 0.4 per cu. ft. while silicone slides exposed at the same time, with one exception, varied from 45.1 to 277.4 per cu. ft. This indicates that very large numbers of fungus spores may be present in the air, but are unable to form colonies when agar plates are used for sampling. This may be due to unsuitable media but the majority of the

fungi are soil types and capable of growth on artificial media. The exceptions are parasites such as the rusts and to some extent the smuts which are not cultivable. It is felt that the chief reason for the difference in numbers of fungus spores on silicone slides and those based on the ability to form colonies on nutrient agar is loss of viability in the air.

References

1. KELLY, C. D., PADY, S. M., and POLUNIN, NICHOLAS. Aerobiological sampling methods from aircraft. Can. J. Botany, 29 : 206–214. 1951.
2. LUCKIESH, M., TAYLOR, A. H., and HOLLADAY, L. L. Sampling devices for airborne bacteria. J. Bact. 52 : 55–65. 1946.
3. PADY, S. M. and KELLY, C. D. Use of silicones in aerobiology. Science, 110 : 187. 1949.
4. PADY, S. M. and KELLY, C. D. Studies on microorganisms in arctic air during 1949 and 1950. Can. J. Botany, 31 : 107–122. 1953.
5. PADY, S. M., KELLY, C. D., and POLUNIN, NICHOLAS. Arctic aerobiology. II. Nature, 162 : 379. 1948.
6. POLUNIN, NICHOLAS, PADY, S. M., and KELLY, C. D. Arctic aerobiology. Nature, 160 : 876. 1947.
7. PROCTOR, B. E. The microbiology of the upper air. II. J. Bact. 30 : 363–375. 1935.
8. WOLFE, FRED T. The microbiology of the upper air. Bull. Torrey Botan. Club, 70 : 1–14. 1943.

STUDIES ON MICROORGANISMS IN ARCTIC AIR DURING 1949 AND 1950[1]

By S. M. Pady[2] and C. D. Kelly[3]

Abstract

The numbers of bacteria and fungi in arctic air were determined by daily sampling at Churchill, Man., during July and August 1950, and in three flights, one to Baker Lake, N.W.T., and the remainder to Resolute Bay, N.W.T. Of the three samplers which were used simultaneously the G.E. Bacterial Air Sampler gave slightly higher readings than the Bourdillon Slit Sampler, while the filter gave low readings throughout.

Daily averages of bacteria at ground level ranged from 0.9 to 30.1 per cu. ft., and in the flight to Resolute Bay from 0.3 to 0.9 per cu. ft. while the comparable readings of the fungi were 0.5 to 4.4 and 0.01 to 0.7 per cu. ft. with the slit sampler. Considerable variation occurred in ground level samples, not only from day to day but throughout the day. Silicone slide studies revealed high numbers, up to 115 per cu. ft., which includes a high proportion of nonviable fungus spores. Most of the organisms are soil inhabiting forms but some fungus parasites were present, chiefly as smut (*Ustilago*) spores. Evidence indicates that winds originating in the south carry large numbers of organisms northward, many of which are nonviable when they reach the arctic, while north winds of polar origin contain very low numbers even in the summer.

Prior to 1947 little was known of the numbers and types of organisms found in the air over arctic regions. In 1933 Meier (5) with the co-operation of Col. Lindbergh, exposed slides principally over Greenland on the latter's trip from the United States to Europe and spores of numerous fungi were obtained. No cultures however were obtained on this trip. In 1947 the senior author prepared agar plates and slides which were exposed by N. Polunin (9) during four flights in the Canadian Arctic. The plates on return to the laboratory had more bacterial colonies than fungi and Dr. C. D. Kelly agreed to study them. After further work a joint project was undertaken[4] on the microbiology of arctic air by Dr. C. D. Kelly, bacteriologist, Dr. S. M. Pady, mycologist, and Dr. N. Polunin, palynologist and continued until Dr. Polunin left McGill in 1950. Quantitative and qualitative methods were intensively studied and sampling techniques worked out for sampling from aircraft (4). Numerous test flights were made over temperate and subarctic regions and the results of these studies are reported separately (3). On Sept. 13, 1948, as part of this project, a sampling flight was made by Polunin over the North Pole using a qualitative method. Later, in March 1949, the authors with Polunin made

[1] *Manuscript received September 19, 1952.*

Contribution from the Department of Botany and Department of Bacteriology, McGill University, Montreal, Que.

[2] *Associate Professor of Botany.*

[3] *Assistant Professor of Bacteriology.*

[4] *The writers gratefully acknowledge the support of the Defence Research Board in providing grants for this work and particularly the help and assistance of Mr. Graham Rowley. Acknowledgment is also made to the Engineering staff of Transport Squadron 426 of the R.C.A.F. for their aid in many phases of the investigation. The authors wish to express their appreciation for the invaluable technical assistance of Sharon Layne, Anna Pawlikowska, and Lucia Kapica.*

a second flight to the North Pole using both quantitative and qualitative methods of sampling. The results have recently been published independently by Polunin (8).

The first quantitative samples of microorganisms under summer conditions in arctic air were taken during a flight from Montreal to Baker Lake, N.W.T., in July 1949, with the R.C.A.F. In 1950, daily ground level samplings were made at Churchill, Man., during July and August. Between Aug. 1 and 3 two flights were made from Churchill to Resolute Bay, N.W.T. This paper reports the results of these studies with particular emphasis on the numbers of bacteria and fungi obtained by standard techniques of air sampling. The data regarding the particular fungi and bacteria involved, their number and prevalence, will be published separately.

PART I.—Flight from Winnipeg, Man., to Baker Lake, N.W.T., 1949*

During this flight, made on July 19, 1949, in a R.C.A.F. Dakota, samples were taken over subarctic and arctic territory. Churchill is located on the 'Tree Line' and the portion of the flight from Winnipeg to Churchill was over subarctic territory while the remainder of the flight to Baker Lake and back to Churchill was over true arctic territory. The McGill G.E. sampler and the filters were installed in the usual manner (4) and exposed simultaneously throughout the flight. For the first 20 min. after take off, the inlet hoses and sampling chamber were scoured with free flowing air, then plates and silicone slides were exposed for 30 min. and filters for 60 min. Eight sets of samples were made with 8 filters, 11 agar plates, 2 silicone slides, and 3 sets of controls.

The numbers of bacteria and fungi are shown in Table I. Except for filters in sets V and VI, which are presumably contaminated, the numbers of bacteria and fungi are approximately equal. The average number of bacteria obtained from all of the filters was 0.5 per cu. ft.; from the plates 0.3 with an over-all average for the entire flight of 0.4 per cu. ft. The fungi obtained from the filters was 0.3 per cu. ft.; from the plates 0.7 per cu. ft. averaging 0.5 per cu. ft.

Of the two silicone slides exposed for fungous spores one gave a reading of 6.0 spores per cu. ft. the other 15.8 per cu. ft. Since the number of fungi in the plates was less than one per cu. ft. it is evident that only a small fraction of the spores which fell on the agar were viable.

Identification was made of some of the 125 fungous colonies which developed in the 11 sets of agar plates exposed by the G.E. method. Of the 63 cultures obtained, 23 were *Cladosporium*, 6 *Alternaria*, 5 *Penicillium*, 3 *Papularia*, 2 *Stemphylium*, 2 yeasts, 2 unknown, and 20 nonsporulating. The slides contained chiefly spores of *Cladosporium*, *Alternaria*, and smuts (*Ustilago*).

Cultures of representative bacteria were made from the Churchill to Baker Lake flight, and also from a short local flight over Churchill. In the Baker

* *Data on the flight to Baker Lake were published by Polunin (8), without our knowledge or consent and the numbers reported for bacteria and fungi are not correct.*

TABLE I

DATA OF SAMPLES TAKEN ON JULY 1948 WITH McGILL G.E. SAMPLER AND FILTER IN R.C.A.F. DAKOTA. ALTITUDE 3000 FT., AIR SPEED 160 M.P.H. SETS I–IV, WINNIPEG TO CHURCHILL, MAN.; V, VI, CHURCHILL TO BAKER LAKE, N.W.T.; VII, VIII, BAKER LAKE TO CHURCHILL

Set	Length of exposure, min.	Total cu. ft. of air	Wind	Temp., °C.	Bacteria		Fungi		
					Filter	G.E. Plates	Filter	G.E. Plates	Slides
i	60	30.6	300/25k.	8°	0.4		0.3		
	30	30	"	8°		0.3		0.7	
	30	30	320/20	4°		0.3		0.4*	
ii	60	34.8	010/22	3°	1.7		0.3		
	60	60	"	3°					6.0
iii	60	36.7	020/15	−3°	0.05		0.05		
	30	30	"	−3°		0.2		1.7	
	30	30	040/10	−2°		0.1		0.6	
iv	56	37.1	040/10	−2°	0.05		0.15		
	30	30	"	−2°		0.2		1.3	
v	60	27.1	040/13	7°	42.9**		0.0		
	30	30	"	7°		1.1		0.1	
	30	30	"	7°		0.3		0.3	
vi	60	41.0	355/10	7°	25.6**		0.3		
	30	30	"	7°		0.3		1.2*	
	30	30	320/15	7°		0.3		0.7*	
vii	60	28.2	350/15	8°	0.2		0.6		
	60	60	"	8°					15.8***
viii	68	59.7	020/15	7°	0.3		0.3		
	30	30	"	7°		0.3		0.5	
	30	30	025/5	6°		0.1		0.7	

* *Fungus spreader.*
** *Filter presumably contaminated.*
*** *High reading due to one large clump of 29 Cladosporium spores.*
NOTE: *3 G.E. plates—controls: 2 col. 0 col. 4 col.*

Lake trip 55 cultures or smears were made: of these 18 were cocci in clusters, 17 sporeformers, 10 cocci in tetrads, 6 Gram-negative rods, and 4 Gram-positive pleomorphic rods. Thirty colonies were identified from the local flight, 18 being Gram-positive pleomorphic rods, 4 cocci in clusters, 3 Gram-negative rods, 3 sporeformers, and 2 cocci in tetrads.

The numbers of microorganisms were rather low owing to the polar air mass in which the samples were taken. The average of one organism per cu. ft. obtained on the flight to Baker Lake is in contrast to the figures obtained on the previous day, July 18, when samples taken between Montreal and Winnipeg gave numbers varying from 2 to 15 per cu. ft., the latter high readings being near Winnipeg, in warm southwesterly air. The flight to Baker Lake was valuable in giving preliminary information as to numbers of organisms and the length of the sample required in arctic air under summer conditions.

II.—Studies at Fort Churchill, Man., in 1950

In July 1950 daily sampling of arctic air was commenced at Churchill, Man. The purpose of this investigation was to obtain a large number of samples in order to determine more accurately the number and if possible the types of bacteria and fungi present in the air. Moreover, it was hoped that with numerous daily samples taken over a longer period that some information would be obtained on the question of correlation of numbers with air masses. A study of meteorological records indicated that while the prevailing winds were N. or NW. with polar air and low temperatures, there were occasional days in the summer with a warm southerly wind. It was decided that the best period for sampling would be mid-July to mid-August. Since daily flights were not possible, arrangements were made to take daily ground level samples and to supplement this work by occasional flights.

Through the co-operation of the D.R.B. Northern Laboratory* under Dr. K. L. Fisher and Sq/Ldr. Harris of the R.C.A.F., the sampling equipment was assembled on a platform atop the old R.C.A.F. hangar about 55 ft. from the ground at the edge of the runway. Three pieces of apparatus were used, the glass wool filter described in an earlier publication (4), the G.E. Bacterial Air Sampler (4), and the Bourdillon Slit Sampler (1) which had not previously been used. Agar plates for the G.E. sampler and the slit sampler, contained either the modified Czapek's agar, with glucose and yeast extract (CGY) or (7) the Czapek's agar used in the original work in 1947 (7), which lacked glucose (CY). Silicone slides were also used, at first in the G.E. sampler but later with the slit sampler. Unfortunately the G.E. sampler became inoperative during the first week, and was not available until August.

Sampling procedure was as follows: a single filter was used each day, being run continuously until between 400 and 500 cu. ft. of air were sampled. The G.E. and the slit sampler were run in parallel series, the exposure times varying from 10 to 45 min., the majority being 30 min. in length. One and sometimes two silicone slides were taken daily in the slit sampler. Controls were used for sterility of the medium and on operational techniques. The agar plates were incubated at 25° C. for five days, at which time counts and identifications were made. The filters were stored at room temperatures and plated out following return to Montreal. The total number of samples was 139 which was made up of 20 filters, 27 silicone slides, 92 agar plates (73 CGY and 19 CY medium) taken in the period between July 21, and Aug. 9, 1950.

Results

The total numbers obtained by the three samplers are shown in Fig. 1. The numbers of bacterial and fungus colonies in the plates were averaged and this figure recorded. There is considerable variation not only from day to day but in the results obtained from the different samplers. While the slit

* *The writers wish to thank those in charge of the Defence Research Northern Laboratory for the use of facilities during this period, particularly the loan of essential equipment. Their co-operation throughout the investigation and in assigning technical assistant Ian Fraser to aid in the work is gratefully acknowledged.*

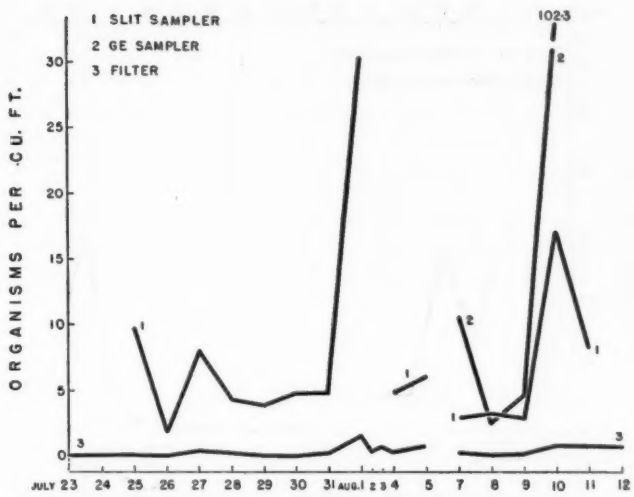


Fig. 1. Total numbers of bacteria and fungi obtained by various sampling devices. Figure is an average of all samples taken during a single day. Colonies grow on Czapeks solution agar. Hangar roof, Churchill, Man. July 23–August 12, 1950.

sampler and G.E. sampler gave comparable results the filter results were uniformly low. This may be due in part to the difficulty in removing all of the organisms from the glass wool fibers during the plating process. Some loss of viability also may have taken place since four to six weeks elapsed between the time of sampling and plating out.

The data on the daily average numbers of fungi are indicated in Fig. 2. On the basis of colony formation fungi were not numerous never reaching 5 per cu. ft. Silicone slides however revealed very high numbers of fungus spores in the air. It is important to note that these readings were from the slit sampler which concentrated the spores and permitted reading under high power of the microscope. On Aug. 4 a high reading of 114.9 fungus spores per cu. ft. was obtained while the colonies by the same sampler varied from 2.2 to 5.9 averaging 3.5 per cu. ft.

Bacteria were generally more numerous than the fungi (Fig. 3) but their numbers varied considerably from day to day. The filter gave very low readings even on days when the other samplers indicated that much higher numbers were present in the air.

Considerable variation in numbers was found in the daily samples taken during any one day. Fig. 4 summarizes the data from 72 samples of fungi and bacteria taken on 15 days by the slit sampler and grown on nutrient agar. The daily minimum and maximum counts for bacteria and for fungi are given as well as the average, since the average does not indicate the wide range of variation usually found. On Aug. 4, for example, consecutive readings taken throughout the day in the slit sampler gave the following numbers: for

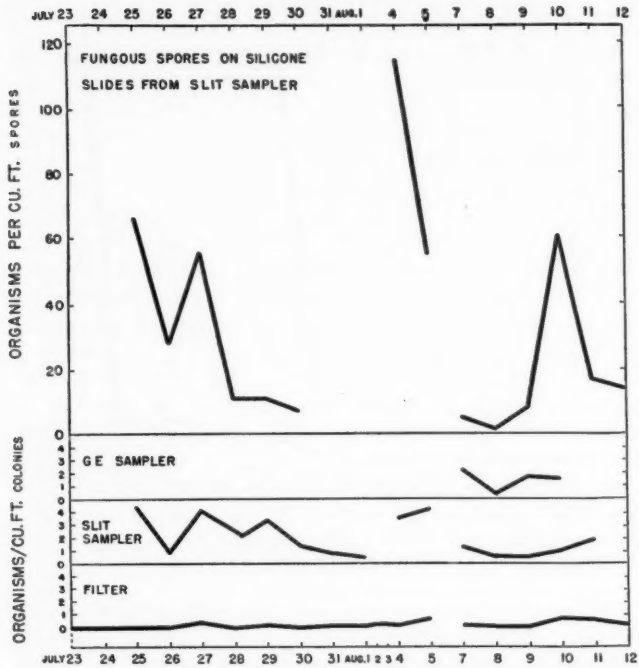

JULY 23 24 25 26 27 28 29 30 31 AUG.1 4 5 7 8 9 10 11 12
FUNGOUS SPORES ON SILICONE SLIDES FROM SLIT SAMPLER
ORGANISMS PER CU. FT. SPORES
120
100
80
60
40
20
0
ORGANISMS/CU.FT. COLONIES
GE SAMPLER
SLIT SAMPLER
FILTER
0 1 2 3 4
JULY 23 24 25 26 27 28 29 30 31 AUG.1 2 3 4 5 7 8 9 10 11 12

FIG. 2. Numbers of fungi obtained by various sampling methods. Upper graph represents total numbers of fungus spores deposited on silicone treated slides: lower graphs represent viable spores which formed colonies after five days' incubation at 25° C.

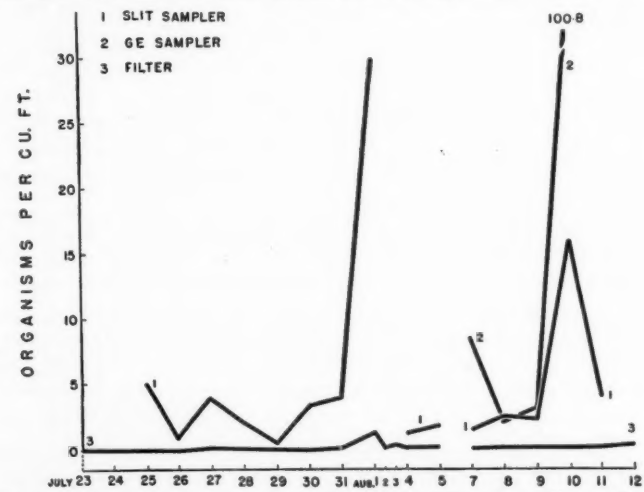


FIG. 3. Average numbers of bacteria obtained by different sampling methods.

bacteria 2.9, 1.4, 2.1, 1.2, 1.4, 0.5, 0.9, 0.4 per cu. ft.; fungi 2.3, 2.2, 3.2, 5.9, 4.3, 4.3, 3.8, 2.3 per cu ft. On certain days such as Aug. 1 the range for bacteria was very wide yet for the fungi very low counts were obtained (Fig. 4). While the range for the fungi was seldom as great as for the bacteria the reverse was occasionally true, as on July 29 (Fig. 4). While there is some suggestion that on certain days the fungi are more numerous in the air between 2:00 and 4:00 p.m. this does not hold true for most sampling days. Sometimes the highest numbers of fungi are found in the morning, sometimes in the afternoon. The highest numbers of bacteria likewise are found at different times on different days. Simultaneous peaks occurred only four times.

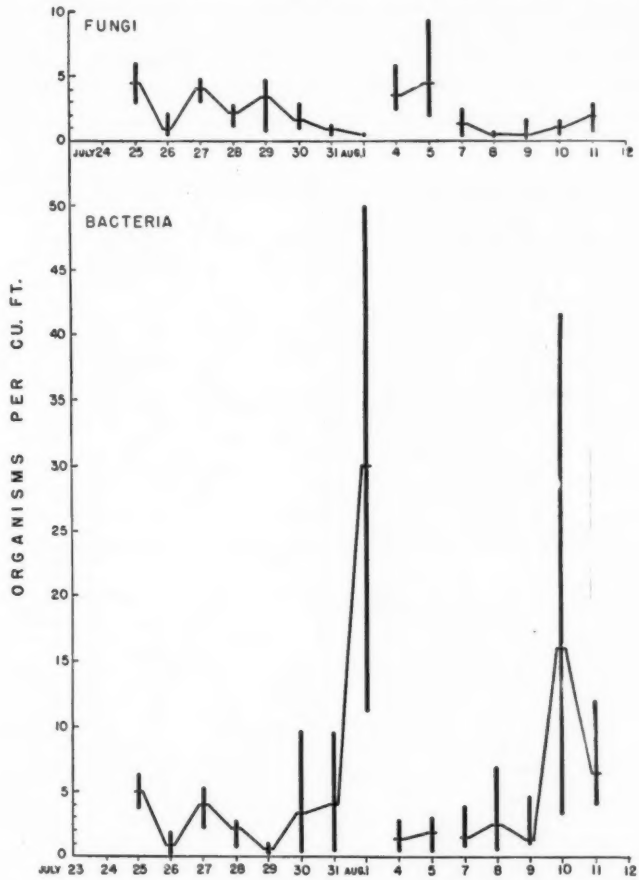


FIG. 4. Maximum, minimum, and average numbers of bacteria and fungi in daily samples with the slit sampler. Extreme variations within one day, see August 1, and variations from day to day are clearly indicated.

III.—Flight to Resolute Bay, N.W.T.

On Aug. 1 a flight was made from Churchill, Man., 1100 miles north, to Resolute Bay, N.W.T. by R.C.A.F. North Star, returning on Aug. 2 via Coral Harbor. A second trip on Aug. 3 was made over the same route. Because the McGill G.E. sampler was unserviceable it was necessary to substitute the slit sampler in its place as an emergency measure. Normally the slit sampler is operated in free air with a large vacuum pump to draw air through the sampling chamber. Since it was not possible to operate the pump in the aircraft it was hoped that the slip stream of the aircraft would provide sufficient air flow. It was necessary to carry the rubber hose from the inlet elbow in the window directly to the slit sampler where it was taped in place. The exit hose led from the manometer gauge to the outlet elbow. It was found that the air flow through the slit was adequate, and a constant air flow, usually of 1 cu. ft. per min. was obtained by an adjustable hose clamp on the exit tube.

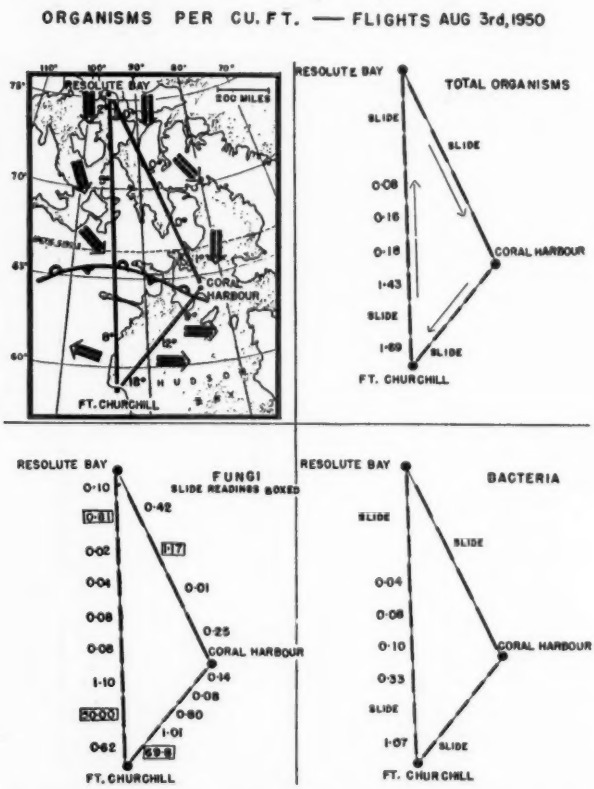


FIG. 5. For explanation see text.

The filter was installed in the usual way (4). In the first flight an accident to the special door, which contained the fittings for the inlets and outlets leading to the samplers, prevented samples being taken from Resolute Bay to Coral Harbor: it was repaired in time to permit sampling from Coral Harbor to Churchill.

The results of the first trip are given in Fig. 5. On the accompanying map the wind direction and air temperatures are given while on the three accompanying figures the location of the sample, the total number of organisms obtained per sample, the number of bacteria and fungi are given, as well as the results of the fungus spore count from the silicone slides in the slit sampler. Warm continental air covered the lower part of Hudson Bay with a wind that varied from southwest to southeast. The approximate position of the front is indicated by the dotted lines on the map (Fig. 5). North of the front the flight was in cool polar air with a north wind.

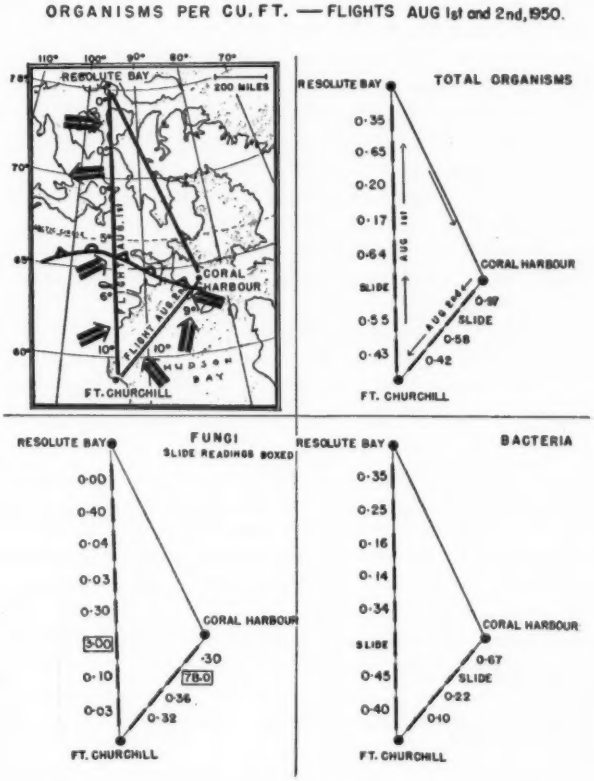


FIG. 6. For explanation see text.

The second flight was completed in one day Aug. 3, under ideal conditions, and the figures for the outgoing and return flights are comparable. The warm air mass had extended somewhat further north than on the previous day and there was a strong flow of cool polar air from the north. The numbers of organisms obtained on this flight are shown in the diagrams on Fig. 6.

The differences in numbers of organisms found in the warm air and in the numbers found in the polar air are clearly indicated. Numbers of bacteria in the cold polar air are very low as compared with the numbers in the warm continental air mass, and the same is true for the fungi. The value of the fungus spore readings is clearly demonstrated here as the polar air has very few spores while the southern air had large numbers of spores.

Fungi and Bacteria in Arctic Air

Although a detailed study of the bacteria and fungi will be published separately it is desirable here to record the principal types that were present in the ground level samples at Churchill and in the samples taken during the flights to Resolute Bay. Bacteria were more numerous than fungi in arctic air in the summer on the basis of the samples reported here. An attempt was made to ascertain the types of bacteria found on the plates by smearing and staining the colonies. At first all the bacterial colonies in a plate were examined but later only those in one sector, usually a quarter of the plate were examined. In this way over 1000 colonies from the plates exposed at Churchill and most of the colonies from the plates from the Resolute Bay flights, were placed in groups on their general morphology and Gram reaction. The results of these identifications are given in Table II. The commonest bacteria in the Churchill plates were Gram-positive pleomorphic rods 46%, with spore formers only 18%, whereas in the Resolute Bay plates sporeformers were more numerous (51%) than the pleomorphic rods. Since only 45 determinations were made in the latter case it is felt that these figures may not be significant. The bacteria are considered to be chiefly soil inhabiting organisms.

TABLE II

TYPES OF BACTERIA OCCURRING IN GROUND LEVEL SAMPLES AT CHURCHILL, MAN., AND IN SAMPLES FROM TWO FLIGHTS TO RESOLUTE BAY, N.W.T., AUGUST 1 AND 3, 1950

Types	Churchill ground level, 19 days' sampling	Resolute Bay, two flights 9000 ft.
Gram-positive pleomorphic rods	502 (46%)	10 (23%)
Gram-negative rods	215 (20%)	0
Sporeforming rods	199 (18%)	22 (51%)
Cocci in clusters and tetrads	157 (15%)	10 (23%)
Miscellaneous	12 (1%)	3 (7%)
Totals	1085	45

TABLE III

TYPES OF FUNGI PRESENT AT CHURCHILL IN GROUND LEVEL SAMPLES. DATA FROM DAILY AVERAGES, COVERING 15 DAYS USING SLIT SAMPLER METHOD. JULY 24–AUGUST 11, 1950

	Colonies on nutrient plates			Fungus spores on silicone slides		
	Number days present	Number per cu. ft.		Number days present	Number per cu. ft.	
		Min.	Max.		Min.	Max.
Cladosporium	14	.06	1.9	14	.4	25.9
Yeasts	15	.01	.7	14	.6	44.0
Penicillium	15	.01	.23	—	—	—
Stemphylium	9	.01	.16	2	.06	.2
Alternaria	5	.01	.07	2	.02	.3
Pullularia	9	.004	.08	—	—	—
Smuts	—	—	—	15	.10	19.5
Rusts	—	—	—	14	.04	2.2
Hyphae	—	—	—	4	.04	.4
Nonsporulating	15	.08	2.7	—	—	—
Total	15	.4	4.4		1.7	114.9

The numbers and kinds of fungi found on the nutrient plates as colonies and as fungus spores on the silicone slides are summarized in Table III. It was possible to determine the numbers per cu. ft. of the chief types both on the plates and on the slides. *Cladosporium* is the commonest fungus in all samples, at times reaching a maximum of 25.9 spores per cu. ft. on a slide. That most of these are nonviable is indicated by the fact that the highest numbers in the nutrient plates was 1.9 per cu ft. On the slides yeasts were more numerous than *Cladosporium*. Smuts (*Ustilago* sp.) rusts and hyphal fragments were present on the slides, the smuts occurring on all slides exposed although never in very high numbers (Table III).

The numbers of fungi obtained at 9000 ft. in the flights to and from Resolute Bay are given in Table IV. The numbers are much lower than those at ground level at Churchill but the same kinds of fungi are present. Numbers varied during different parts of the flight. This is clearly shown in Fig. 6. Samples of fungi taken in the warm air of southerly origin had high numbers while those taken in the cool polar air were very low.

The use of the silicone slides in the slit sampler proved to be a valuable technique since it permitted a reading of the total deposit of spores under high power. It is clear that while there may be few viable fungus spores in the arctic air, larger numbers may be carried northward than revealed by the samplers using nutrient media and colonies to obtain counts.

Fungus spores may provide the best evidence for correlation with air masses. At Churchill meteorological data were obtained for all samples and attempts were made to determine the nature of the air mass involved. In many cases it was difficult to determine the type of air mass being sampled. In Table V the data from 15 days' sampling have been arranged according to maximum

TABLE IV

TYPES OF FUNGI OBTAINED IN TWO FLIGHTS FROM CHURCHILL TO RESOLUTE BAY AND RETURN. SLIT SAMPLER USED IN R.C.A.F. NORTH STAR, 9000 FT. AUGUST 1–3, 1950. DATA FROM ACTUAL NUMBER OF PLATES (24) AND SLIDES (6) THAT WERE EXPOSED

	Colonies on nutrient plates			Fungous spores on silicone slides		
	Times present	Number per cu. ft.		Times present	Number per cu. ft.	
		Min.	Max.		Min.	Max.
Cladosporium	15	.01	.67	6	.17	8.2
Yeasts	3	.03	.11	6	.12	8.2
Penicillium	6	.02	.07	—	—	—
Stemphylium	2	.05	.08	1	.01	—
Alternaria	10	.02	.15	1	.02	—
Pullularia	9	.02	.12	—	—	—
Smuts	—	—	—	6	.12	8.2
Rusts	—	—	—	6	.02	3.0*
Hyphae	—	—	—	3	.01	.67
Nonsporulating	17	.02	.83	—	—	—
Average		.2	1.3		.81	78.0

* *Many moss spores were present in these slides and some were undoubtedly missread as rust spores.*

TABLE V

CORRELATION OF NUMBERS OF FUNGUS SPORES ON SILICONE SLIDES WITH TEMPERATURE. ONE SLIDE EXPOSED DAILY EXCEPT FOR JULY 26, AUGUST 10, AUGUST 11, WHEN TWO, FOUR AND SIX SLIDES RESPECTIVELY WERE AVERAGED. SPORES FROM SILICONE SLIDES FROM SLIT SAMPLER. EXPOSURE LENGTH MOSTLY 30 MIN. AT 1 CU. FT. PER MIN.

Date	Air mass	Wind	Maximum daily temp.	Spores per cu. ft.	Average
Aug. 4	T ?	SW.	84	114.9	
July 21		ESE.	78	37.7	
" 25	Pp mod.	SW.	76	66.0	
" 27	P mix.	SW./E.	68	56.1	52.8
Aug. 5	Pp mod.	NW./SW.	67	55.3	
" 10	Pp mod.	Var.	68	61.1	
" 11		NW./NE.	65	17.7	
" 12	P mod.	W.	65	14.2	
July 30	P mod.	SE.	63	7.2	
Aug. 7		E.	60	5.3	
July 29		SE.	58	11.2	
Aug. 9		NNW.	56	8.3	10.4
July 28	P mar.	SE.	53	11.1	
" 26	Pp	NNE.	51	28.8	
Aug. 8	Pc	NNW.	47	1.7	

temperature attained. On the days when temperatures were highest, the wind was usually southerly and numbers of spores high. The average spore load for the day when maximum temperatures were from 64 to 84° F. was 52.8 per cu. ft., as compared with 10.4 per cu. ft. when the temperatures were lower.

On most sampling days only one silicone slide was exposed, and the figure thus obtained may not be typical for that day. When more than one slide was used variations were encountered. This is illustrated in the readings obtained on Aug. 11 when six slides were exposed (Table VI). On the previous day there was a reading of 61.1 spores per cu. ft. The first slide on Aug. 11 had fairly high numbers but numbers were low following the cool front which passed through at 11:30 a.m. (Table VI).

TABLE VI

DAILY VARIATION IN NUMBERS OF FUNGUS SPORES SAMPLED THROUGH A SINGLE DAY, AUGUST 11, 1950, ON SILICONE SLIDE IN THE SLIT SAMPLER. RATE OF DEPOSITION 1 CU. FT./MIN. AT 11:30 A.M. A COLD FRONT PASSED OVER CHURCHILL. WIND NW. CHANGING TO NE.

Slide No.	Time	Temp., F°.	Exposure	Fungus spores per cu. ft.
1	1017	65°	30 min.	38.9
2	1210	60–55°	136 "	7.4
3	1435	54°	10 "	15.0
4	1448	53°	20 "	19.4
5	1511	53°	30 "	11.7
6	1544	53°	60 "	8.6

Other Biological Materials

Moss spores were found on silicone slides from slit sampler taken during the flights from Churchill to Resolute Bay and return, and moss protonema grew in many plates. On Aug. 2, between Coral Harbor and Churchill many moss spores were collected on the slides, and it was calculated that there were 27.9 moss spores per cu. ft. On the following day en route to Resolute Bay, and on the return trip readings of 4.5 and 7.6 moss spores per cu. ft. were obtained. The slides at Churchill had very few moss spores except on Aug. 10 and 11. However, 11 filters yielded protonemata when they were plated out.

Pollen grains were found on all but three of the silicone slides exposed at ground level at Churchill. The highest numbers occurred on Aug. 10 when 6.5 pollen grains per cu. ft. were collected, the same day that moss spores were abundant. Low numbers were taken on the following day when 0.04 pollen grains per cu. ft. were determined while on Aug. 12 pollen grains were completely absent. Data on the pollen grains will be published with the results of the silicone slides and other samplers in a paper on the arctic fungi.

Discussion

The quantitative sampling methods that were worked out for determining the numbers of bacteria, fungi, and other microorganisms on a cubic foot basis are satisfactory, but the modern high speed aircraft is far from ideal as a sampling station. Speeds of over 200 m.p.h. do not permit many exposures: in the arctic, or in winter air, where numbers are low, a long exposure is required and the number of samples is greatly limited. On the other hand good air flow is obtained without the use of pumps, meteorological data are available, and the samples can be accurately located from the navigators log. The flights were made in service aircraft of the R.C.A.F. on scheduled trips, and the flight direction and altitude were not always the best for sampling purposes. The flight to Resolute Bay on Aug. 3 fortunately provided ideal conditions for obtaining data on the numbers of organisms carried by two different air masses.

Continuous sampling at ground level in the arctic not only permitted many daily samples to be taken but provided information as to the efficiency of the samplers. The Bourdillon slit sampler and the G.E. sampler gave comparable results but unfortunately the latter was unserviceable during most of the time the work was being carried on at Churchill. The filter gave the lowest readings and appeared to be very inefficient under the conditions in which it was used. The long sample of 300–400 cu. ft. of air, may have been so heavily loaded that the amount of recovery from the glass wool fibers was low. Moreover the delay of many weeks before the filters were processed could have resulted in considerable loss of viability.

In the aircraft the G.E. sampler is used in a special sampling chamber, the McGill G.E. sampler (4). For the trips to Resolute Bay and return it was necessary to utilize the slit sampler which had never been previously used in an aircraft. The data for this trip are not strictly comparable with the data from other flights where the McGill G.E. sampler is used. It is very important in any sampling from aircraft that the method used, particularly the installation of the apparatus be fully and completely described.

In 1947 numerous cultures of *Sporormia* were obtained from the most northerly exposures and it was suggested that this fungus may be truly arctic in origin (6). Spores of *Sporormia* were fairly common on the silicone slides exposed at Churchill. To determine whether these fungi are present locally, rabbit dung was collected and stored in a dry condition. During the winter this material was placed in a moist chamber and numerous perithecia of two species of *Sporormia* developed. It would appear that some local organisms therefore are caught by ground sampling techniques. In order to get into the upper air currents in large numbers, fungi or bacteria must be present in quantity either as parasites on plants and animals or as saprophytes in soil as Gregory has recently pointed out (2). Since soil as such is not abundant in the arctic the chief source of locally produced organisms would be parasites on arctic plants, such as the willow rusts. Most of the bacteria and fungi which grew in the plates were typical soil organisms and it is felt that the

majority of organisms in the arctic air have originated in the agricultural areas to the south and have been carried in to the arctic by southerly winds. It has been pointed out that the number of fungus spores on warm days usually with southerly winds was three to five times higher than on those days when the temperatures were lower and wind was generally from the north.

The use of silicone slides to obtain a sample of all of the fungus spores in the air is a valuable supplement to the techniques employing colony formation as a measure of the number of organisms. Nutrient plates provide a medium where viable spores and bacteria may grow. Nonviable fungus spores and spores whose nutritional requirements were not met by the medium would not be represented by colonies. Silicone slides thus provide a comparison between the fungi capable of growth on nutrient agar and the total actually present in the air. On Aug. 8 the temperatures were 44–47° and numbers of fungi on plates were 0.6, 0.4, 0.04 per cu. ft. by the slit, G.E., and filter samplers, while the fungus spores on the silicone slides indicated 1.7 per cu. ft. On Aug. 10, however, with a southerly wind and temperatures of 53–68°, fungi on plates were 1.4, 1.5, 0.7 per cu. ft. by the same samplers while the silicone slide reading was 92.8 fungus spores per cu. ft. The indications are that large numbers of fungus spores are carried northward but are no longer viable when they reach the arctic, or are unable to grow on the particular medium used. Some evidence for loss of viability is furnished by the omnipresent *Cladosporium*, which grows well on our media, being present in 82% of the plates, and on all but one slide. The highest number of viable *Cladosporium* spores as based on colonies which developed in the plates was 1.9 per cu. ft. whereas on silicone slides a concentration of 8.2 per cu ft. was recorded. The silicone slides also show that many fungi, particularly *Cladosporium*, are in clumps: on nutrient agar these would form but a single colony, hence numbers based on agar are probably low. The presence in the air of groups of spores has also been recently pointed out by Gregory (2).

A striking feature of the fungi which developed in the plates was the prevalence of nonsporulating colonies. At Churchill where many samples were taken daily the number of nonsporulating colonies with the slit sampler varied from 10 to 75% and averaged 51%. Nonsporulating colonies from the filters which were plated out later on return to the laboratory ranged from 11 to 95%, averaging 57%. It is difficult to explain why air transit should have this effect upon fungus spores, and further studies on this problem are being planned.

The bacteria in northern air are more numerous than the fungi whereas in southern air the reverse is generally true, at least during the summer. During the passage of bacteria through the air there is undoubtedly considerable loss in viability, just as in the case of fungus spores. Unfortunately this cannot be checked as there is no method at present for the direct determinations of bacteria in the air. The only data from this present study that would indicate loss of viability is that the ratio of sporeformers to nonsporeformers is greater

in arctic air than in nonarctic air. The indications are that exposure affects first the Gram-positive pleomorphic rods and Gram-negative rods, followed by the cocci, with larger numbers of sporeforming rods surviving. However, the data here are too scanty or incomplete, and much more work must be done before any conclusions can be drawn as to the kinds of bacteria associated with specific air masses.

The most evident feature of the microorganism population, as found in samples taken at ground level is the great fluctuation which takes place not only from day to day, but during the day. This is especially evident in the numbers of fungi whose spores are large enough to permit counts being made directly from silicone slides. It is also true for bacteria, moss spores, and pollen grains, and indicates that the air may carry much higher numbers of microorganisms than those indicated by conventional methods. There is some correlation with numbers of bacteria and fungi at Churchill and temperatures and, it is strongly suspected, a correlation with air masses and it is believed that a detailed knowledge of the origin and trajectory of air masses would reveal a correlation between the specific air masses and numbers and types of microorganisms. The arctic is not an ideal place to seek such data since the air is predominately polar in origin and tropical air masses rarely reach beyond Churchill. The investigation of the microorganisms of the air is being continued on a year round basis from the roof of the Sun Life Building in Montreal in order to determine not only the loads carried by winds at various seasons of the year but to ascertain, if possible, the types of microorganisms carried by specific air masses.

References

1. BOURDILLON, L. B., LIDWELL, O. M., and THOMAS, JOHN C. A slit sampler for collecting and counting air-borne bacteria. J. Hyg. 41 : 197–224. 1941.
2. GREGORY, P. H. Fungus spores. Brit. Mycol. Soc. Trans. 35 : 1–18. 1952.
3. KELLY, C. D. and PADY, S. M. Microbiological studies of air over some nonarctic regions of Canada. Can. J. Botany, 31 : 90–106. 1953.
4. KELLY, C. D., PADY, S. M., and POLUNIN, NICHOLAS. Aerobiological sampling methods from aircraft. Can. J. Botany, 29 : 206–214. 1951.
5. MEIER, F. C. Collecting microorganisms in the arctic atmosphere with field notes and material by C. A. Lindberg. Sci. Monthly, 40 : 5–20. 1935.
6. PADY, S. M. Fungi isolated from arctic air in 1947. Can. J. Botany, 29 : 46–56. 1951.
7. PADY, S. M., KELLY, C. D., and POLUNIN, NICHOLAS. Arctic aerobiology II. Preliminary report on fungi and bacteria isolated from the air in 1947. Nature, 162 : 379–381. 1948.
8. POLUNIN, NICHOLAS. Seeking airborne particles about the North Pole. Svensk. Botan. Tid. 45 : 320–354. 1951.
9. POLUNIN, NICHOLAS, PADY, S. M., and KELLY, C. D. Arctic aerobiology. Nature, 160 : 876. 1947.

A FURTHER SURVEY OF PLANTS FOR ANTIBACTERIAL SUBSTANCES[1]

By R. E. MacDonald[2] and C. J. Bishop[3]

Abstract

One hundred and seventy-seven Nova Scotian plants were tested for antibacterial activity using *S. aureus* and *E. coli* as test organisms. Of the plants tested, 33.3% were active, 32.2% against *S. aureus* and 5.1% against *E. coli*. No relationship was found between the activity of the extracts and their pH. The plants tested are listed together with their degree of activity. Among those with the most active extracts were *Alnus crispa*, *Drosera intermedia*, *Erigeron canadensis*, *Hypericum* spp., and *Agrimonia* spp.

Introduction

In folk medicine, plants have long held a leading place as a source of medicinal drugs. For this purpose the early settlers of this continent cultivated herbs, many of which they brought with them from Europe. In recent years various researchers have undertaken systematic studies of the flowering plants in an attempt to find substances with high antibacterial activity which could be used therapeutically in man. A complete review of these studies has been made by MacDonald (10). These researches have been confined for the most part to United States and Europe. In Canada a limited survey was conducted by Panisset and Louis-Marie in 1945, but other than this no work on the problem was reported until Bishop and MacDonald (1) made a survey of 208 Nova Scotian Plants. The present report is a continuation of that survey.

It was noted in reviewing the work of various authors that few had tried any solvent other than water for extracting substances from plants and only Bushnell, Mitsuno, and Nakinodan (3) had made any effort to determine the relationships between the pH of water extracts and the activity of these extracts. With these considerations in view a survey was undertaken in the summer of 1951 to study the antibacterial activity of various native and introduced plants of Nova Scotia.

Materials and Methods

Twenty milliliters of nutrient agar of the following composition:

Bacto Peptone	10.0 gm.
Sodium chloride	5.0 gm.
Bacto Beef Extract	3.0 gm.
Bacto Yeast Extract	2.5 gm.
Bacto Agar	18.0 gm.
Water	1000.0 ml.

[1] *Manuscript received October 20, 1952.*
Contribution from the Department of Biology, Acadia University, Wolfville, N.S. This project received financial assistance from the National Research Council of Canada and the Nova Scotia Research Foundation. This paper is based on part of a thesis submitted to Acadia University by R. E. MacDonald in partial fulfillment of the requirements for the degree of Master of Arts.
[2] *Research Assistant. Present address—University of Western Ontario, London, Ontario.*
[3] *Research Professor of Biology, Acadia University, Wolfville, N.S.*

at a pH of 7.4, was pipetted into a sterile Petri dish and allowed to harden. One milliliter of an undiluted 18 hr. broth culture of the test organism (*Staphylococcus aureus* No. 209 P or *Escherichia coli* A.T.C.C. No. 9637) was poured on the surface of the agar and spread by tipping the dish so that the entire agar surface was covered. The excess broth culture was then drained off, and the dish and cover inverted in the incubator for one-half hour in order to dry.

After the plates were dried, the glass tops were replaced with metal covers containing fiber absorption disks (Brewer Petri Metal Tops). These were found to be far superior for assay purposes because they readily absorbed the excess moisture, and also reduced breakage. Four or five glass penicylinders (outside diameter 8 mm. × 10 mm. high) were placed in each Petri dish and filled with the various solutions to be tested. After standing at room temperature for one-half hour the plates were placed in the incubator. Results were recorded after 18 hr. of incubation.

The plants were prepared for extraction by macerating 20 gm. of the plant material in 50 ml. of water in a Waring blendor. The slurry was then strained through cheesecloth and squeezed to dryness. The pH of the filtrate was recorded. The residue was divided into four equal parts and each part was placed in a small sized mortar. To each portion was added 5 ml. of ethanol, ethyl ether, acetone, or the aqueous filtrate. After grinding the plant residue for three to five minutes in the solvent with a pestle, the solvent was removed and placed in a micro test tube for storage until assay.

The plants tested were collected mainly in the Annapolis Valley and Cape Breton Island. They were identified according to the eighth edition of Gray's Manual of Botany (6), with the exception of the Genus *Malus*, the members of which were identified according to Rehder (12).

Results

One hundred and seventy-seven plants were tested and are listed in the following tabulation. Those marked with an asterisk showed activity against one or both organisms and are recorded together with their degree of inhibition, in Table I. Fifty-nine (33.3%) of the plants tested were active, 57(32.2%) against *S. aureus* and nine (5.1%) against *E. coli*, seven being active against both organisms.

There was no evidence that the pH of the aqueous extract of the plant had any bearing on its activity or on the activity of the other extracts of the same plant. Neither the most acid plant (*Vitis labrusca*, pH 2.9) nor the least acid plant (*Symphytum asperum* pH 6.2) showed any activity.

The use of several solvents gave a considerably higher percentage of activity than could be obtained with the use of only one solvent. This was also noted by Carlson and Douglas (4) and by Bishop and MacDonald (1).

A comparison of the plant list in the tabulation with all the plants previously reported in the literature, numbering some 2200 (10), shows that antibacterial activity is present in two families not previously reported (Cistaceae and

Juncaceae). One hundred twenty-seven species of the plants tested in this survey have not previously been reported tested, and of those previously recorded as not active, seven were found to be active in this survey. It should be pointed out, however that many of the plants previously tested are not listed in the literature because they were found inactive.

LIST OF PLANTS TESTED FOR ANTIBACTERIAL ACTIVITY

Family	pH
Aceraceae	
**Acer pensylvanicum* L.	5.1
**A. rubrum* L.	4.9
**A. saccharum* Marsh.	3.9
**A. spicatum* Lam.	3.9
Aizoaceae	
Mollugo verticillata L.	5.5
Alismaceae	
Alisma triviale Pursh.	5.4
Aquifoliaceae	
Nemopanthus mucronata (L.) Trel.	5.0
Araceae	
Acorus calamus L.	5.3
Araliaceae	
**Aralia racemosa* L.	5.3
Balsaminaceae	
Impatiens capensis Meerb.	4.6
Betulaceae	
**Alnus crispa* Pursh. var. *mollis* (Ait.) Fern.	—
**Betula papyrifera* Marsh.	4.8
**B. populifolia* Michx.	5.2
Boraginaceae	
Myosotis laxa Lehm.	6.2
M. scirpioides L.	6.2
Symphytum asperum Lepechin	6.2
Campanulaceae	
Campanula rapunculoides L.	5.3
Cannabinaceae	
**Humulus lupulus* L.	5.2
Caprifoliaceae	
**Lonicera canadensis* Marsh.	5.6
**Triosteum aurantiacum* Bickn.	5.2
**Viburnum alnifolium* Marsh.	4.7
V. cassinioides L.	4.7
V. edule (Michx.) Raf.	5.9
Cistaceae	
**Hudsonia ericoides* L.	3.9
**Lechea intermedia* Leggett	4.4

Family—*Continued*	pH
Compositae	
Achillea ptarmica L.	5.7
**Anthemis cotula* L.	5.1
**A. tinctoria* L.	5.4
Artemisia Stelleriana Bess.	5.1
**Aster nemoralis* Ait.	5.5
**Centaurea nigra* L.	5.3
**Erigeron canadensis* L.	5.7
Galinsoga ciliata (Raf.) Blake	5.4
Gnaphalium sylvaticum L.	5.4
Hieracium vulgatum Fries.	5.2
**Lapsana communis* L.	5.9
Senecio jacobaea L.	4.7
**S. viscosus* L.	5.1
**Solidago macrophylla* Pursh	5.0
Convolvulaceae	
Convolvulus sepium L.	5.4
Cruciferae	
Berteroa incana (L.) DC.	5.5
Cardamine pratensis L. var. *palustris* Wimm. & Grab.	6.0
Dentaria diphylla Michx.	5.7
Cupressaceae	
**Juniperus horizontalis* Moench	5.8
**Thuja occidentalis* L.	—
Cyperaceae	
Carex intumescens Rudge	4.8
Carex lurida Wahlenb.	5.6
C. stipata Muhl.	5.9
Dulichium arundinaceum (L.) Britt.	4.6
Eleocharis obtusa (Wild.) Schultes	5.9
Scirpus atrocinctus Fern.	5.1
S. rubrotinctus Fern.	5.7
Droseraceae	
**Drosera intermedia* Hayne	3.9
Empetraceae	
Corema conradii Torr.	—
Ericaceae	
Arctostaphylos uva-ursi (L.) Spreng.	4.5
**Gaylussacia baccata* (Wang.) K. Koch	3.5
**Rhododendron* sp.	4.0
**Vaccinium corymbosum* L.	5.0
V. macrocarpon Ait.	3.4
V. vitis-idaea L. var. *minus* Lodd.	4.5

* *See Table I.*

LIST OF PLANTS TESTED FOR ANTIBACTERIAL ACTIVITY—*Continued*

Family—*Continued*	pH
Eriocaulaceae	
Eriocaulon septangulare With.	5.3
Fagaceae	
Fagus grandifolia Ehrh.	5.2
Fumariaceae	
Dicentra cucullaria (L.) Bernh.	6.0
Gentianaceae	
Bartonia paniculata (Michx.) Muhl. var. *intermedia* Fern.	—
Nymphoides cordata (Ell.) Fern.	5.0
Gramineae	
Agrostis scabra Willd.	5.4
A. tenuis Sibth.	5.4
Avena sativa L.	5.6
Brachelytrum erectum (Schreb.) Beauv.	5.2
**Glyceria striata* (Lam.) Hitchc.	4.9
G. canadensis (Michx.) Trin.	5.4
Hordeum jubatum L.	5.6
Leersia oryzoides (L.) Sw.	5.5
Muhlenbergia uniflora (Muhl.) Fern.	5.4
Phleum pratense L.	6.1
Phragmites communis Trin. var. *Berlandieri* (Fourn.) Fern.	5.1
**Spartina pectinata* Link	5.4
Haloragidaceae	
Myriophyllum tenellum Bigel.	—
M. verticillatum L.	—
Hydrocharitaceae	
Elodea canadensis Michx.	—
Hypericaceae	
**Hypericum canadensis* L.	4.5
**H. virginicum* L. var. *fraseri* (Spach) Fern.	4.0
Iridaceae	
**Sisyrinchium montanum* Greene var. *crebrum* Fern.	5.2
Isoetaceae	
Isoetes macrospora Dur.	4.7
Juncaceae	
Juncus articulatus L.	5.6
J. effusus L.	5.7
**J. stygius* L.	—
**Luzula multiflora* (Retz.) Lejeune	5.3
Labiatae	
Mentha piperita L.	6.0
Nepeta cataria L.	5.7
Satureja vulgaris (L.) Fritsch var. *neogaea* Fern.	6.0
Lentibulariaceae	
Utricularia cornuta Michx.	—
U. geminiscapa Benj.	—
U. minor L.	—
Liliaceae	
Clintonia borealis (Ait.) Raf.	5.0
Maianthemum canadense Desf.	—
**Medeola virginiana* L.	4.8
Polygonatum pubescens (Willd.) Pursh	6.0
Lycopodiaceae	
Lycopodium inundatum L.	4.6
Lythraceae	
Lythrum salicaria L.	4.8
Malvaceae	
Malva neglecta Wallr.	5.6
Nymphaeaceae	
**Brasenia schreberi* Gmel.	4.5
**Nuphar variegatum* Engelm.	3.9
Onagraceae	
Circaea canadensis Hill	3.6
Orchidaceae	
Corallorhiza maculata Raf.	5.1
**C. trifida* Chatelain var. *verna* (Nutt.) Fern.	—
Goodyera oblongifolia Raf.	—
Habenaria macrophylla Goldie	4.6
H. sp.	4.9
Osmundaceae	
Osmunda cinnamomea L.	5.4
O. claytoniana L.	5.6
O. regalis L. var. *spectabilis* (Willd.) Gray	5.3
Papaveraceae	
**Sanguinaria canadensis* L.	4.5
Pinaceae	
Pinus resinosa Ait.	—

* *See Table I.*

LIST OF PLANTS TESTED FOR ANTIBACTERIAL ACTIVITY—*Concluded*

Family—*Continued*	pH
Polygonaceae	
**Polygonum cuspidatum* Sieb. & Zucc.	4.8
Polypodiaceae	
Athyrium filix-femina (L.) Roth	5.5
**Dennstaedtia punctilobula* (Michx.) Moore	5.0
Dryopteris noveboracensis (L.) Gray	5.0
D. spinulosa (O. F. Muell.) Watt. var. *intermedia* (Muhl.) Underw.	5.0
Polypodium virginianum L.	5.1
**Polystichum acrostichoides* (Michx.) Schott	5.1
**P. braunii* (Spenner) Fee var. *purshii* Fern.	5.1
P. lonchitis (L.) Roth	5.1
Pteridium aquilinum (L.) Kuhn var. *latiusculum* (Desv.) Underw.	5.4
Potamogetonaceae	
Potamogeton pectinatus L.	6.1
Pyrolaceae	
**Pyrola rotundifolia* L.	4.9
**P. secunda* L.	4.9
Ranunculaceae	
Ranunculus reptans L.	—
Rhamnaceae	
**Rhamnus frangula* L.	5.0
Rosaceae	
**Agrimonia gryposepala* Wallr.	4.9
**A. striata* Michx.	4.9
Crataegus macrosperma Ashe	4.4
Cydonia oblonga Mill.	5.1
Filipendula ulmaria (L.) Maxim.	5.2
**Geum macrophyllum* Willd.	4.9
Malus atrosanguinea Schneid.	4.7
**M. prunifolia* Borkh.	3.4
M. toringo Sieb.	4.9
**M. toringoides* Hughes	6.0
**M. zumi* Rehd. var. *calocarpa* Rehd.	4.0
Potentilla anglica Laicharding	5.1
P. norvegica L.	5.1
Prunus armeniaca L.	4.3
P. avium L.	6.2
P. cerasus L.	6.4
**P. domestica* L.	4.0
P. persica Batsch.	4.2
P. virginiana L.	5.1
Pyrus communis L.	5.0
**Rubus odoratus* L.	5.0
Sanguisorba canadensis L.	5.2

Family—*Concluded*	pH
Rubiaceae	
Galium kamtschaticum Steller	5.0
G. palustre L.	5.0
G. triflorum Michx.	5.0
G. verum L.	5.0
Salicaceae	
Populus grandidentata Michx.	—
P. tremuloides Michx.	—
Salix pentandra L.	4.6
**S. purpurea* L.	5.0
Scrophulariaceae	
**Melampyrum lineare* Desr.	5.4
Mimulus moschatus Dougl.	—
Veronica americana (Raf.) Schwein.	5.3
V. scutellata L.	5.4
Schizeaceae	
Schizaea pusilla Pursh	5.0
Selaginellaceae	
Selaginella selaginoides (L.) Link	—
Solanaceae	
Capsicum annuum L.	5.3
**Datura stramonium* L.	5.3
Sparganiaceae	
Sparganium eurycarpum Engelm.	4.9
Ulmaceae	
Ulmus glabra Huds.	5.4
Umbelliferae	
Heracleum sphondylium L.	5.6
Pastinaca sativa L.	5.4
Sanicula marilandica L.	5.6
Urticaceae	
Urtica gracilis Ait.	6.0
U. procera Muhl.	5.5
Violaceae	
Viola septentrionalis Greene	—
Vitaceae	
Vitis labrusca L.	2.9
Xyridaceae	
Xyris montana Ries	5.0

* *See Table I.*

TABLE I

PLANTS WHOSE EXTRACTS SHOWED ANTIBACTERIAL ACTIVITY

Plant	pH	*S. aureus*				*E. coli*			
		*1	2	3	4	1	2	3	4
Aceraceae									
Acer pensylvanicum	5.1	—	12	13	—	—	—	—	—
A. rubrum	4.9	—	12	16	—	—	27	—	—
A. saccharum	3.9	—	12	17	—	—	—	—	—
A. spicatum	3.9	11	14	16	—	—	—	—	—
Araliaceae									
Aralia racemosa	5.3	—	18	—	—	—	—	—	—
Balsaminaceae									
Impatiens capensis	4.6	—	14	15	—	—	—	—	—
Betulaceae									
Alnus crispa	—	—	20	18	19	—	—	—	—
Betula papyrifera	4.8	—	13	11	—	—	—	—	—
B. populifolia	5.2	—	—	13	12	—	—	—	—
Cannabinaceae									
Humulus lupulus	5.2	15	18	18	16	—	—	—	—
Caprifoliaceae									
Lonicera canadensis	5.6	—	11	13	—	—	—	—	—
Triosteum aurantiacum	5.2	—	11	13	—	—	—	—	—
Viburnum alnifolium	4.7	—	—	—	—	—	—	—	10
Cistaceae									
Hudsonia ericoides	3.9	—	13	15	—	—	—	—	—
Lechea intermedia	4.4	11	sl	12	—	—	—	—	—
Compositae									
Anthemis cotula	5.1	—	—	12	—	—	—	sl	—
A. tinctoria	5.4	—	—	12	—	—	—	—	—
Aster nemoralis	5.5	—	13	—	—	—	—	—	—
Centaurea nigra	5.3	—	14	15	11	—	—	—	—
Erigeron canadensis	5.7	—	20	21	10	—	—	13	—
Lapsana communis	5.9	—	—	12	—	—	—	—	—
Senecio viscosus	5.1	—	15	12	11	—	—	—	—
Solidago macrophylla	5.0	—	13	13	—	—	—	—	—
Cupressaceae									
Juniperus horizontalis	5.8	—	12	—	—	—	—	—	—
Thuja occidentalis	—	—	—	—	—	15	—	—	—
Droseraceae									
Drosera intermedia	3.9	14	18	21	23	—	sl	12	22
Ericaceae									
Gaylussacia baccata	3.5	—	17	15	sl	—	—	—	—
Rhododendron sp.	4.0	—	—	12	—	—	—	—	—
Vaccinium corymbosum	3.0	12	14	15	—	10	12	12	—
Gramineae									
Glyceria striata	4.9	—	16	—	—	—	—	sl	—
Spartina pectinata	5.4	—	—	—	—	10	—	—	—

* 1. *Water extract.* 2. *Alcohol extract.* 3. *Acetone extract.* 4. *Ether extract.*

NOTE: *Figures given are the diameters of the zones of inhibition measured in millimeters.*

TABLE I—*Concluded*

PLANTS WHOSE EXTRACTS SHOWED ANTIBACTERIAL ACTIVITY—*Concluded*

Plant	pH	*S. aureus*				*E. coli*			
		*1	2	3	4	1	2	3	4
Hypericaceae									
Hypericum canadensis	4.5	—	20	18	15	—	—	—	—
H. virginicum var. *fraseri*	4.0	—	25	12	17	—	—	—	—
Iridaceae									
Sisyrinchium montanum var. *crebrum*	5.2	—	10	12	—	—	—	—	—
Juncaceae									
Juncus stygius	—	—	10	—	—	—	10	—	—
Luzula multiflora	5.3	—	—	10	—	—	—	—	—
Liliaceae									
Medeola virginiana	4.8	—	16	13	13	—	—	—	—
Nymphaeaceae									
Brasenia schreberi	4.5	—	10	12	—	—	—	—	—
Nuphar variegatum	3.9	—	11	11	—	—	—	—	—
Orchidaceae									
Corallorhiza trifida var. *verna*	—	—	—	12	—	—	—	—	—
Papaveraceae									
Sanguinaria canadensis	4.5	—	17	19	10	—	—	—	—
Polygonaceae									
Polygonum cuspidatum	4.8	—	—	10	—	—	10	10	—
Polypodiaceae									
Dennstaedia punctilobula	5.0	sl	—	12	—	—	—	—	—
Polystichum acrostichoides	5.1	—	—	—	11	—	—	—	—
P. braunii var. *purshii*	5.1	—	—	10	—	sl	—	—	—
Pyrolaceae									
Pyrola rotundifolia	4.9	—	—	11	10	—	—	—	—
P. secunda	4.9	—	14	14	—	—	—	—	—
Rhamnaceae									
Rhamnus frangula	5.0	—	13	15	—	—	—	—	—
Rosaceae									
Agrimonia gryposepala	4.9	sl	19	19	19	—	—	—	—
A. striata	4.9	14	27	26	19	—	—	—	—
Geum macrophyllum	4.9	—	—	11	—	—	—	—	—
Malus prunifolia	3.4	11	11	13	—	—	10	—	—
M. toringoides	6.0	11	13	16	—	—	—	—	—
M. zumi var. *calocarpa*	4.0	—	—	12	—	—	—	—	—
Prunus domestica	4.0	14	—	—	—	—	—	—	—
Rubus odoratus	5.0	—	13	15	—	—	—	—	—
Salicaceae									
Salix purpurea	5.0	sl	12	13	12	—	—	—	—
Scrophulariaceae									
Melampyrum lineare	5.4	—	—	11	—	—	—	—	—
Solanaceae									
Datura stramonium	5.3	12	15	14	—	—	—	—	—

* 1. *Water extract.* 2. *Alcohol extract.* 3. *Acetone extract.* 4. *Ether extract.*

NOTE: *Figures given are the diameters of the zones of inhibition measured in millimeters.*

Discussion

The degree of inhibition should not be the important consideration in evaluating plants for antibacterial substances, since the method employed is of necessity one of qualitative rather than quantitative value. To say that one extract is more active than another using the results of a survey of this sort would be entirely without basis. Some antibacterials are present only in minute amounts in the plant, others may be nearly insoluble in the solvents used, and still others even in high concentrations may be only slightly active. Various substances, too, may be present in the plant which inhibit the activity of the active substance greatly.

The results of the present survey are in agreement with those found by Bishop and MacDonald (1). However, while the percentage of the plants active was the same (33.3% compared with 33.4%) the degree of activity for each extract was considerably less. This is thought to be due to several variations made in technique. In the present survey the pH of the media was adjusted to 7.4, yeast extract was added to stimulate the growth of the test organism, and the alcohol, ether, and acetone extracts were each made on fresh samples of ground plant material. In the previous survey on the other hand, the pH was unadjusted and remained at 6.5–6.8, no growth stimulant was added, and the alcohol, ether, and acetone extracts were made in sequence on the same plant material. This indicates that while no difference in over-all results will be found, more marked inhibition will be produced by using media similar to that used in the earlier survey.

The antibacterial substances in plants often occur in certain organs of the plants and not in others. Osborn (11) found that the active principle from *Crepis taraxicifolia* was present in the leaves, but was activated only by an enzyme present in the flowers. Southam (13) found Thujaplicin to be present only in the heartwood of red cedar, and Cavallito, Bailey, and Kirchner (5) found the active principle of burdock to occur "almost exclusively in the green leaves". Other workers have found the active principles distributed throughout the plants.

Sometimes the antibacterial component will withstand drying as is the case with *Arctium minus*, *Sanguinaria canadensis* (7) and *Asarum europeum* (11), frequently it is destroyed as is the case with *Ranunculus* sp. (11) or *Pyrus malus* (2). The recognition of the presence of an antibacterial substance in a plant will thus be influenced by the stability of the substance and its distribution in the plants in which it occurs.

The function of these antibacterial substances in plants is not known, Little and Graubaugh (9) could find no relationships between disease resistance and presence of antibacterial extracts, but Irving (8) suggested that some such relationship may exist in tomato plants. Until more conclusive work is carried out, however, the reasons for the presence of these substances will remain obscure.

While preliminary surveys of the plants are indispensable, before long all the common plants in a given locality will have been tested, and if any value

is to be gained from the initial survey an attempt must be made to isolate and purify the active substances from the plants possessing them. Only then can the full value of the antibacterial principle become known, and only by doing this can it be hoped that any new substances with possible therapeutic application will be found.

Acknowledgments

The authors are indebted to Dr. E. C. Smith and party for assistance in collection of plant specimens, and to Mr. D. E. Erskine for identification of certain plants.

References

1. BISHOP, C. J. and MACDONALD, R. E. A survey of higher plants for antibacterial substances. Can. J. Botany, 29 : 260–269. 1951.
2. BLIGH, E. G. An antibiotic principle from apple leaves. I. Isolation. II. Properties. Masters Thesis, Acadia University. 1951.
3. BUSHNELL, O. A., MITSUNO, F., and NAKINODAN, T. The antibacterial properties of some plants found in Hawaii. Pacific Sci. 4 : 167–183. 1950.
4. CARLSON, H. J. and DOUGLAS, H. G. Screening methods for determining antibiotic activity in higher plants. J. Bact. 55 : 235–240. 1948.
5. CAVALLITO, C. J., BAILEY, J. H., and KIRCHNER, F. L. The antibacterial principle of *Arctium minus*. I. Isolation, physical properties and antibacterial action. J. Am. Chem. Soc. 67 : 948–950. 1945.
6. FERNALD, M. L. Gray's manual of botany. 8th ed. American Book Co., New York. 1950.
7. HAYES, L. E. Survey of higher plants for presence of antibacterial substances. Botan. Gaz. 108 : 408–414. 1947.
8. IRVING, G. W. The significance of tomatin in plant and animal disease. J. Wash. Acad. Sci. 37 : 293–296. 1947.
9. LITTLE, J. E. and GRAUBAUGH, K. K. Antibiotic activity of some crude plant juices. J. Bact. 52 : 578–591. 1946.
10. MACDONALD, R. E. The presence of antibiotic substances in higher plants with special reference to local species. Masters thesis, Acadia University. 1952.
11. OSBORN, E. M. On the occurrence of antibacterial substances in green plants. Brit. J. Exptl. Path. 24 : 227–231. 1943.
12. REHDER, A. Manual of cultivated trees and shrubs. The MacMillan Company, New York. 1927.
13. SOUTHAM, C. M. Antibiotic activity of extract of Western Red Cedar heartwood. Proc. Soc. Exptl. Biol. Med. 61 : 391–396. 1946.

PRELIMINARY STUDIES OF ROOT AND BASAL STALK ROT OF MATURING CORN IN ONTARIO[1]

By W. E. McKeen[2]

Abstract

In Ontario, basal stalk rot of corn occurs in the autumn and is of major importance, whereas bacterial stalk rot is of no economic significance. The latter disease, which results from stomatal penetration by *Erwinia dissolvens*, may occur on a few susceptible corn plants in early July during extremely hot humid weather, although ordinarily temperatures are sufficiently low in the province to limit its development. Common basal stalk rot of corn is usually initiated by the spread of organisms from diseased corn roots up into the stalks; rotting may begin also in tunnels produced by corn borers. *Diplodia zeae* and *Gibberella zeae*, the two pathogens that are responsible for most of the stalk rotting in the central part of the American corn belt, were of no practical importance during the last two years. Bacteria, *Pythium arrhenomanes*, and *Fusarium moniliforme* were commonly found in the necrotic part of the corn stalk. Diseased plants begin to appear in mid-August and daily become more numerous until the first killing frost occurs or the plants become senescent. The leaves suddenly droop and wither, and are dead within four or five days after the first symptoms appear. By this time, the basal part of the stalk is necrotic and at least some of the roots are decayed. Pith tissues are destroyed and the stalk frequently breaks over near the ground level. All stalk rot pathogens finally produce the same syndrome. *P. arrhenomanes* causes a root necrosis at first which later spreads up into the stalk and, in Ontario, is of major importance in the corn stalk rot disease. *Pratylenchus pratensis* has been found in corn roots but its importance is unknown.

Introduction

For the last two decades approximately 600,000 acres of corn have been grown in Ontario and yields have been consistently below those expected by plant breeders, agronomists, and farmers. In the late 'thirties' the plant breeders believed that they had solved this problem when they introduced hybrid corn, but even the hybrid varieties have continued to decline. This degeneration has never been clearly defined, but it has gradually become so serious that everyone concerned with corn production realizes the urgent need for its elucidation and rectification. Pathologists have been convinced for some time that it is a disease complex, but, because of its elusive nature, no intensive aetiological study was undertaken.

In 1949 and 1950, extensive field surveys proved that basal stalk rot was present in every corn field in Ontario and that it was of major importance. In addition to causing indirect losses arising from the rotting of some of the ears which fall on the ground when the stalks collapse (Fig. 9), this disease has a marked deleterious effect on the yield and quality of the grain produced by affected plants. In slightly infected plants or in plants in which rotting of the tissues has not been extensive until the ears are fairly well matured, the grain is shrunken and of inferior quality. In severely infected plants, however,

[1] *Manuscript received for publication May 1, 1952, and, as revised October 10, 1952.*

Contribution No. 1173 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] *Associate Plant Pathologist, Laboratory of Plant Pathology, Saanichton, B.C.; formerly of Harrow, Ont.*

the ears are very small, and the grain is small and often loosely attached to the cob. In individual plants, it has been estimated that the potential grain yield of an infected plant was reduced by upwards of from 40 to 50%.

Not only does this disease impair the quality of the grain, but it also retards its rate of maturity and renders it more susceptible to other infections. The ears infected with *Fusarium*, *Diplodia*, or other ear rotting organisms are unavoidably mixed with the healthy ears in storage cribs and serve as infection foci, with the result that extensive rotting may be caused in storage by even weak parasites if the ears are not dry.

The present study was undertaken to determine the identity and relative importance of the causal organisms responsible for root and basal stalk degeneration in maturing corn and to study the factors influencing the incidence and severity of this disease in Ontario.

Bacterial Stalk Rot

In Ontario, stalk rot of corn occurs every autumn, but an unusual type of stalk rot appeared in July of 1949. The latter was caused by the bacterium *Erwinia dissolvens* Rosen. This type of disease frequently occurs in the central and southern part of the United States (13) but was much more prevalent there than usual in 1949.

The outbreak of the bacterial stalk rot in Ontario in 1949 was detected in only one inbred, Number 13 (105W × *hy*), at the Experimental Station, Harrow, and all the neighboring inbreds remained healthy. A rotting of the stalk, usually at the first internode (Fig. 2) or first node above ground level, was the first indication of the disease observed. Necrosis usually extended

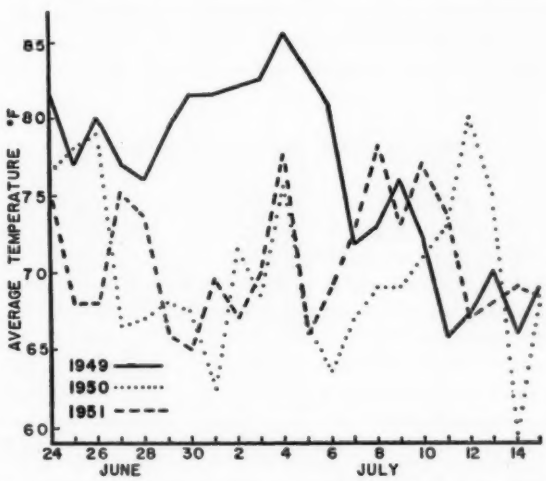


FIG. 1. The average daily temperature in degrees Fahrenheit from June 24 to July 15 for the years 1949–1951.

through the whole thickness of the stalk (Fig. 3), but never extended more than two or three inches in a longitudinal direction. Even after the plants toppled over, the leaves and the remainder of the plant still had a very healthy appearance. No leaf infection was noted.

The rotted portion of a stalk is a dark-brown or blackish, soft, putrid, sunken mass, with abundant transparent bacterial ooze between the vascular strands and beneath the leaf sheath. The parenchyma tissue is attacked, and within two or three days it is completely destroyed and only the vascular strands remain (Fig. 4).

Twenty-three isolations from diseased plants yielded consistently a whitish yellow bacterium on nutrient agar. This bacterium was rod shaped, with an average measurement of 0.6 μ by 1.0 μ (Fig. 5). It was Gram-negative, nonacid-fast, and proved pathogenic on corn. It produced equal parts of hydrogen and carbon dioxide in nutrient broth containing dextrose, but none in the absence of dextrose.

At the end of 22 hr. in nutrient broth at 6°, 12°, 20°, 30°, 35°, 40°, 43°, and 45° C., no growth had occurred at either 6° or 45° C. and very little at 12° C. Fair development occurred at 20° and 40° C., but best development between 30° and 40° C., and, in this range, the liquid was extremely murky and cloudy and a heavy precipitate formed after 22 hr.

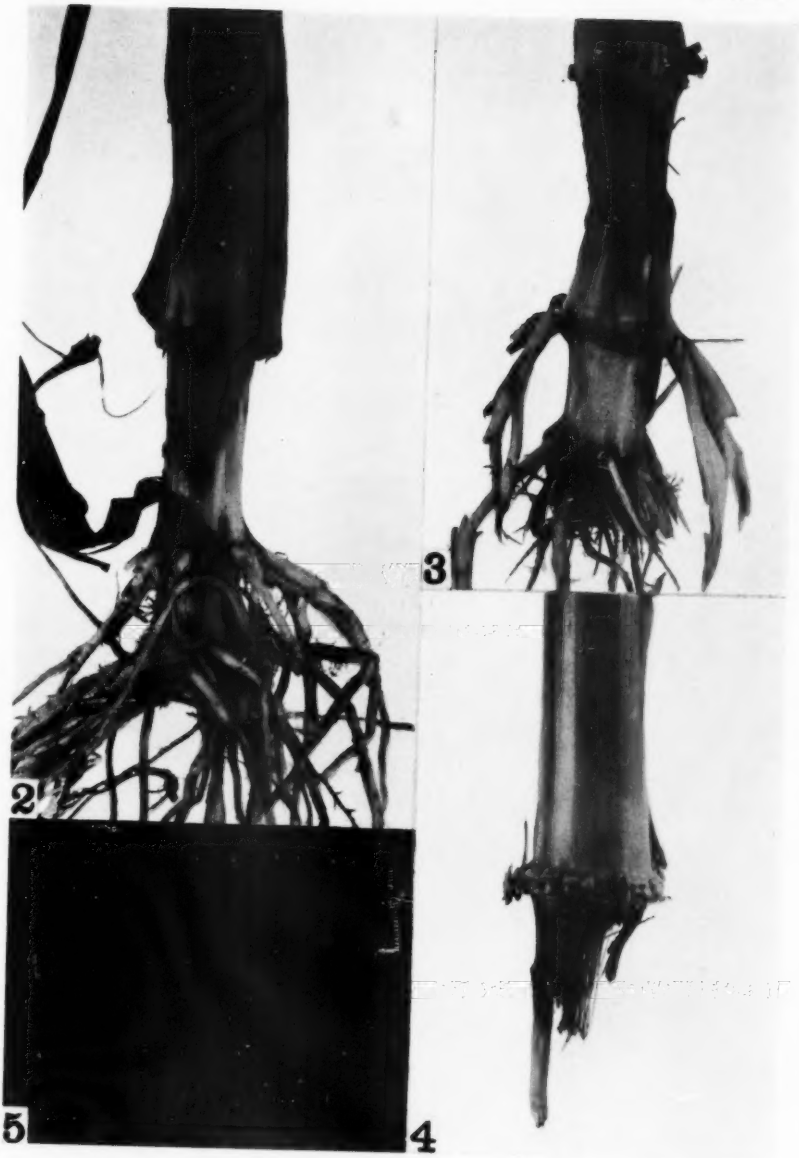
This organism is pathogenic only at high temperatures and it is at such temperatures that it makes the most rapid growth. The fact that bacterial stalk rot occurred only after a prolonged heat period in 1949 and that infection can be obtained only under excessively high greenhouse temperatures substantiates this fact. In Fig. 1, the average daily temperatures are shown for the years 1949, 1950, and 1951, from June 20 to July 15. During this period, precipitation was slightly above normal in all three years. In 1949, bacterial stalk rot began on July 10 after some extremely hot weather, but during the two succeeding years the temperature was considerably lower and no rot occurred.

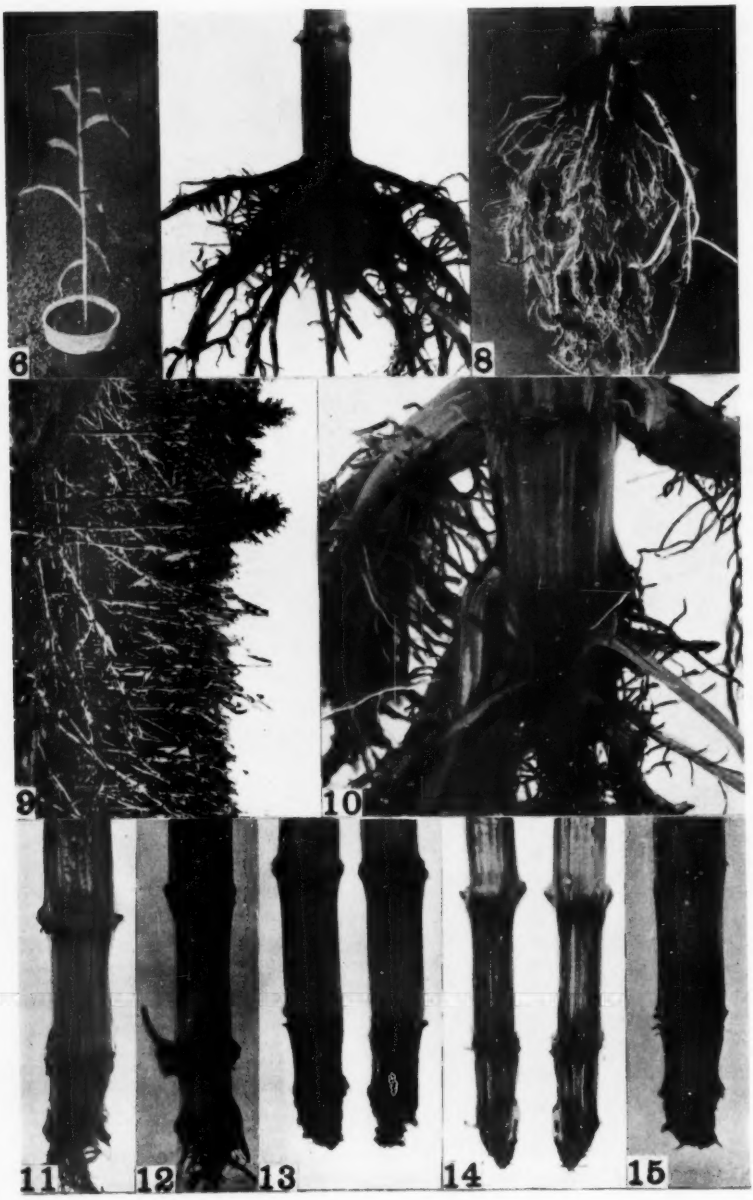
Common Root and Basal Stalk Rot

Symptoms

Aboveground symptoms of root and basal stalk rot may appear from the latter part of August until the first killing frost or until the plant becomes senescent. At first there is a sudden drooping and drying of the leaves (Fig. 6), simulating frost injury, and within four or five days the leaves are dead. By this time the basal part of the stem is necrotic and a dark and water-soaked lesion may appear approximately two inches above the ground (Figs. 7 and 8). Soon all the roots become soft, pink, and hollow. The lesion extends up the stalk several inches, and the stalk becomes very soft and frequently breaks

FIGS. 2–5. FIG. 2. A young bacterial lesion on a corn stalk. FIG. 3. A typical bacterial lesion. It has spread completely across the stem but only about two inches in a longitudinal direction. FIG. 4. The remains of the lesioned end of a corn stalk. The pith has been rotted away, leaving only the fibrovascular bundles. FIG. 5. Rod shaped *Erwinia dissolvens* bacteria.


2
3
4
5


6
8
9
10
11
12
13
14
15

over at this region. Frequently open-pollinated corn plants and occasionally hybrid plants which are suffering from root necrosis are uprooted by strong winds (Fig. 9).

If the stalks are cut open two or three weeks before any external symptoms are present, it will be noticed that the crown and lower nodes are dark brown in color (Fig. 10). Soon black bundles begin to appear in the lower internodes (Fig. 11) and they become more numerous from day to day. A little later, such bundles can be seen through the epidermis (Fig. 12). The pith becomes water-soaked and then pinkish in color (Figs. 13, 14, and 15). A pink discoloration, which has commonly been used as an indicator for the presence of *G. zeae*, follows afterwards, and finally the pith is rotted away and only the fibrovascular bundles and the sclerous outer tissues remain (Fig. 16).

Initiation of this disease is correlated with the ontogeny of the host. If a susceptible variety is planted at weekly intervals in the spring, a short interval occurs between the appearance of the first disease symptoms in each plot in the autumn. The disease always appears in the oldest plants first.

Corn varieties show a range of susceptibility; for example, Canada hybrid 531 is extremely susceptible, but the male parent is more resistant than the female parent. The inbred L.C.19 is completely susceptible and, because of this, it was used in the aetiological studies that were carried out.

Aetiological Studies

Numerous isolations made from L.C.19 stalks from two to three weeks previous to the first appearance of stalk rot yielded bacteria only. Bacteria were found throughout the whole stem and were very abundant in the black bundles. However, further investigations showed that bacteria are present in other corn inbreds and hybrids, even as early as two days after the seed germinated. They apparently do no damage, at least not in the young growing plants.

In the autumn, typical stalk rot symptoms never appear until fungi are present in the host tissue. Although *Rhizoctonia solani*, *Rhizopus nigricans*, *Penicillium* spp., *Aspergillus* spp., and *Gibberella zeae* have occasionally been isolated, *Pythium arrhenomanes* and *Fusarium moniliforme* were the only two

---

FIGS. 6–15. FIG. 6. A corn plant showing the first stalk rot symptoms. The leaves are drooped and slightly withered. FIG. 7. The basal part of a corn plant having necrotic roots and a lesion on the first internode above the ground. FIG. 8. The root system of a maturing plant with a few necrotic roots and a tiny lesion on the internode. FIG. 9. An inbred which, owing to root necrosis, was uprooted by the wind. Another inbred in the adjacent plot is standing well, thus indicating less susceptibility. FIG. 10. The basal part of a corn plant split longitudinally to show the brownness of the crown and first node. FIG. 11. The basal part of a corn stalk showing black fibrovascular bundles scattered throughout the pith. FIG. 12. A corn stalk with black bundles showing through the epidermis. FIG. 13. A corn stalk split longitudinally to show a young lesion. FIG. 14. Portions of two diseased corn stalks showing the first two internodes above ground level; decay has progressed less in the upper than in the lower internode in which empty spaces are beginning to appear. Fig. 15. A stalk with the lesion centered around the first node above the ground. The central part of the first internode is mostly healthy.

fungi which were usually present in the necrotic tissue. Their frequency of isolation is given in Table I. All isolations were made before necrosis had spread above the second or third internode.

TABLE I

FREQUENCY OF ISOLATION OF *P. arrhenomanes* AND *F. moniliforme* FROM DIFFERENT REGIONS OF 100 DISEASED L. C. 19 INBRED PLANTS

Region of isolation	*F. moniliforme*	*P. arrhenomanes*
Crown	62	2
Second internode	29	14
Third internode	18	48

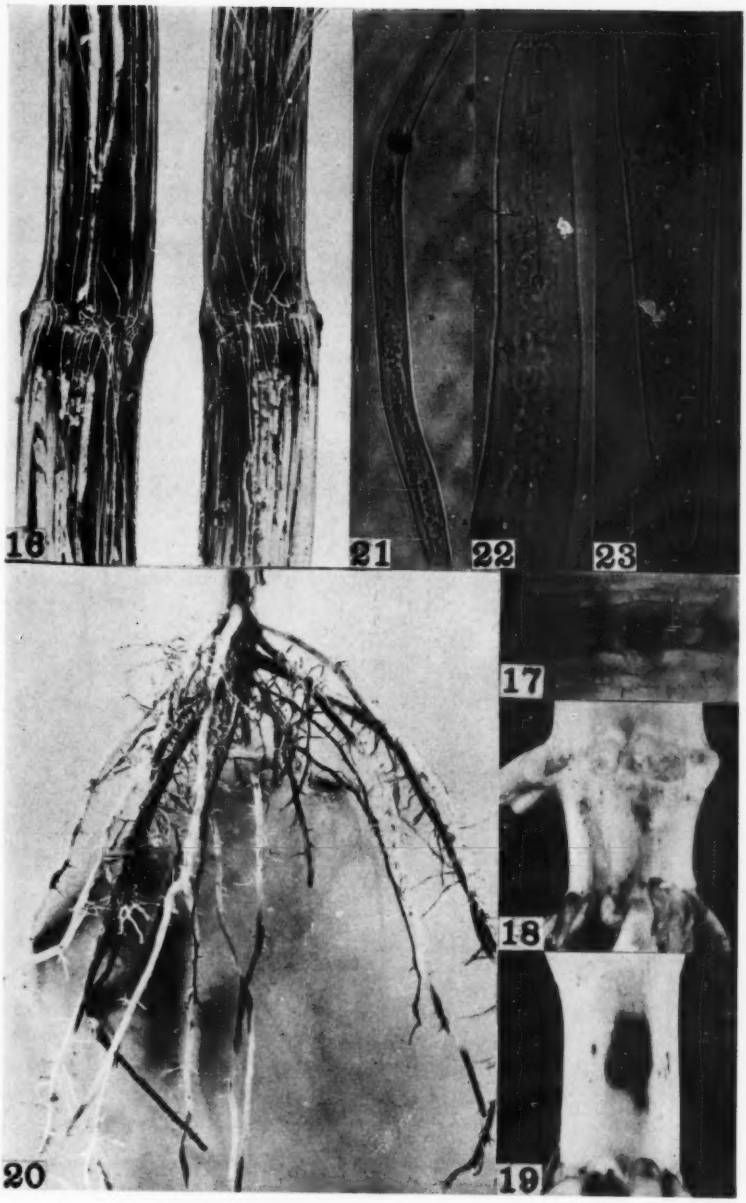
Table I shows that *F. moniliforme* was very abundant in the crown and second internode, while *P. arrhenomanes* was most frequently obtained from the third internode. It was observed that *P. arrhenomanes* was found only in the upper brown, water-soaked part of the lesion, and on no occasion was it obtained from the lower pinkish region where the *F. moniliforme* mycelium was abundant. Apparently this *Pythium* is quickly destroyed or overrun by bacteria or fungi which follow it. Only very occasionally did *F. moniliforme* appear at the top of the lesion without *P. arrhenomanes* being present. If isolations are made after the destruction of the pith, *P. arrhenomanes* cannot be obtained, but millions of *F. moniliforme* spores are clustered on the fibro-vascular bundles.

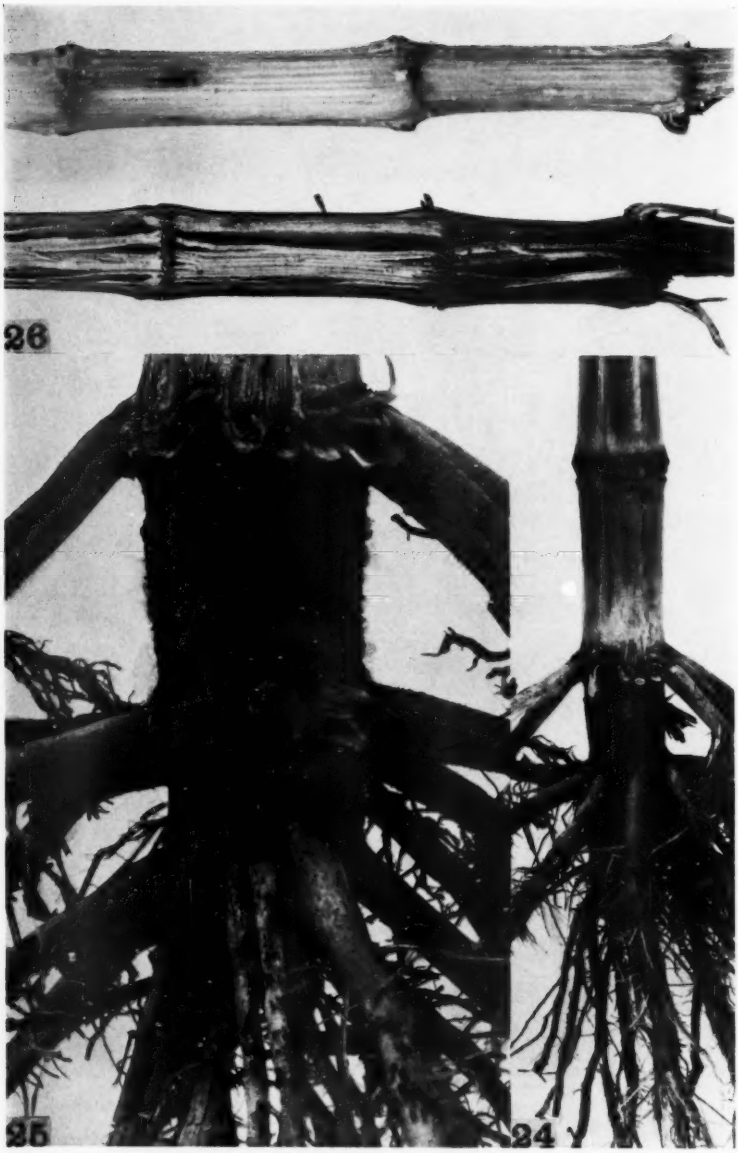
Although stalk rot appears somewhat later in commercial hybrids than in inbreds, the same organisms are obtained. From 42 commercial hybrid plants which were suffering from stalk rot, *P. arrhenomanes* was found in 26 of them.

Sometimes in the inbred L. C. 19 bacteria enter the stomata in the epidermis of the first or second internode (Fig. 17) and small necrotic areas result (Figs. 18 and 19). *F. moniliforme* makes use of the lesions as portals of entry, and stalk rot may occasionally arise in this manner.

Previous work by the author (12) has shown that *P. arrhenomanes* causes a seedling root rot of corn and it seemed feasible that this organism might be causing the root rot that occurred in maturing plants, since it was frequently

FIGS. 16–23. FIG. 16. A corn stalk split longitudinally to show the final result of the rot; most of the pith has been rotted away. FIG. 17. A section of epidermal tissue from a lower internode in which the guard cells of a stoma and two of the adjoining cells on either side are infected with bacteria. FIG. 18. Dark and water-soaked bacterial spots on a lower internode. FIG. 19. A rather large bacterial lesion on a basal internode. FIG. 20. A root system of a large corn plant having several lesions on its roots. FIG. 21. *P. pratensis;* spear knobs and vulva can be seen. FIG. 22. The anterior end of *P. pratensis.* The dorsal esophageal gland orifice appears closely behind the knobs. FIG. 23. The striae on the posterior end of *P. pratensis* can be observed; vulva and anus are also present.


16
21
22
23
17
18
19
20


26
25
24

found in decaying stalks. In commercial varieties and usually in L.C.19, necrosis begins in one or several of the adventitious or primary roots and spreads up into the stalk (Fig. 20). In order to determine the causal organism, numerous isolations during various stages of maturity were made from maturing plant roots grown in three stalk rot soils. Table II shows that *P. arrhenomanes* was frequently present in the roots taken from the three types of soil and was obtained in 73% of the total isolations. This pathogen must have been missed several times in the isolations, because it usually could be detected by microscopic observation in all the diseased roots. It has been observed that cool fall weather increases the amount of *P. arrhenomanes* root rot and ultimately the amount of stalk rot.

TABLE II

FREQUENCY OF ISOLATION OF *P. arrhenomanes* FROM DISEASED CORN ROOTS AT VARIOUS STAGES OF MATURITY GROWN IN DIFFERENT SOIL TYPES

Source and type of soil	Age of plants in days	No. of platings	Frequency of *P. arrhenomanes*
King's Brookston clay loam	74	35	22
" " " "	78	44	39
" " " "	87	30	29
" " " "	92	23	18
" " " "	94	16	15
Winter's Brookston clay loam	74	21	8
" " " "	94	53	49
" " " "	95	19	17
Brian's sandy loam	71	44	15
" " "	91	42	35
" " "	95	30	28
		357	275

Corn plants inoculated with *P. arrhenomanes* by the toothpick method (19) show typical stalk rot symptoms. The possibility of a difference in the susceptibility and resistance of various inbreds to *P. arrhenomanes* is being investigated.

Nematodes

Since stalk rot frequently begins as a root rot, it was felt that perhaps parasitic nematodes were responsible in part for the initial root degeneration. Corn roots were collected and cut into fine pieces with scissors. From these pieces, nematodes were collected by the Baermann funnel method (6). Only

FIGS. 24–26. FIG. 24. The basal part of a corn plant infected with *D. zeae*. The pycnidia are most numerous near the node. FIG. 25. Pycnidia with protruding spore horns. FIG. 26. One-half of the basal portion of each of two corn stalks split longitudinally. Above, interior of a healthy half; below, interior of a diseased half, showing severe damage to the pith by *D. zeae*.

TABLE III

THE LENGTH AND PROPORTIONS OF 15 FEMALE NEMATODES PARASITIC ON CORN ROOTS

	Number designating nematode														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Total length, mm.														
	369	525	525	326	440	525	469	525	525	483	511	540	526	483	497
	Proportions														
*a**	16.6	22.0	18.8	15.5	18.5	23.4	20.9	22.1	22.1	19.1	19.1	21.4	18.7	20.3	22.2
b	7.5	8.3	9.1	5.2	9.0	12.1	7.4	7.5	10.5	6.9	6.9	6.4	6.2	5.7	5.9
c			22.1		20.9	23.1	22.3	20.9	25.0	19.1	18.2	19.2	18.7	18.2	16.9
v	77.7	78.4	78.4		80.6	82.5	81.8	83.8	83.8	79.4	77.7	76.3	77.7	79.6	77.1

* *a = length/greatest breadth; b = length/length of oesophagus; c = length/length of tail, i.e. anus to tip of tail; v = distance of vulva from anterior end, expressed as a percentage of the total length.*

the nematodes provided with a spear were mounted in lactophenol on a slide and cleared by heating over a flame. They were then stained by adding lactophenol containing a very small amount of cotton blue and warmed until suitably stained.

There appeared to be only one species of spear nematode among the numerous other species. The cuticle of the parasitic nematode was marked by distinct transverse striae which extended completely around the terminus of the female tail and were about 1μ apart. Knobs of the spear were almost one-third as wide as the head, and the dorsal esophageal gland orifice (Fig. 21) was about 2 μ behind the spear base. The esophageal glands formed a large lobe (Fig. 22) which extended well over the anterior end of the intestine in which three nuclei could be observed. The posterior uterine branch of the female reproductive system extended one-third to one-half of the distance back to the anus. The lengths and the proportions given in Table III conform to those given by Thorne (15) for *Pratylenchus pratensis* (de Man) Filipjev (Fig. 23). As shown in Table III, there was very little variation in the proportions given for *a*, *c*, and *v*, but in those for *b* the variation was considerable, owing to the fact that it was extremely difficult to determine exactly the point at which the esophagus ended. Representatives of the species were not very numerous, but a few were found on each corn root. No search was made for the *Trichodorus* nematode which was reported in Florida by Christie and Perry (4).

Diplodia zeae Stalk Rot

Diplodia zeae stalk rot was found only in one corn field in Ontario in the autumn of 1949 and did not occur the next season even in the field in which it was present the previous year. Apparently the climatic conditions were not suitable for its development on the stalks because abundant inoculum was present on several ears that were rotting with this organism.

Diplodia zeae stalk rot was readily detected during the latter part of August by its black fruiting bodies which were most numerous near the nodes (Fig. 24), but they could be found also on both the above and below ground roots. Under humid conditions the pycnidia produced long spore horns which could be readily observed on the infected plants (Fig. 25). Two or three weeks after the first symptoms of disease the central part of the stalk had been rotted away (Fig. 26), and the stalks appeared similar to ones which had been destroyed by other stalk-rotting organisms.

Discussion

In Ontario, stalk rot may be readily separated into three types, common basal stalk rot, *D. zeae* stalk rot, and bacterial stalk rot. The first is of major importance, but the last two are uncommon and of no economic significance.

Bacterial stalk rot is caused by *Erwinia dissolvens*, a pathogen that requires an extremely high temperature and humidity to initiate disease. In some of the central and southern corn belt States the temperature remains excessively high for much longer periods than in southern Ontario, and consequently severe

outbreaks may occur in these States whereas plants remain free from disease in Ontario. In the corn belt States the bacteria *Phytomonas lapsa* (2) and *Pseudomonas alboprecipitans* (8 and 9) also may cause bacterial stalk rot, but they are of sporadic occurrence.

Common basal stalk rot, which always occurs in a large percentage of the plants, is very destructive in the autumn and apparently the causal organisms are different from the pathogens that cause stalk rot in the central part of the corn belt of the U.S.A. In Illinois (11), Indiana (17), and Iowa (14) *D. zeae* causes a large amount of the rotting, but *G. zeae* frequently follows closely in second place. Edwards (5) and Wenholz (18) reported that, although this disease could be caused by one or other of several different parasitic fungi, it was almost invariably due to *Gibberella saubinettii*. In 1949, according to Andrews and Jones (1), unusually heavy breakage of corn stalks in Michigan was due to *Fusarium*. In Ontario, *G. zeae* stalk rot is practically nonexistent, even although wheat, which acts as a host to this pathogen, is commonly grown in this area. However, it is not absent from this province, for it frequently causes corn ear rot and scab of wheat. A pink discoloration of the roots and internal part of the necrotic stalk does not necessarily indicate that *G. zeae* is present, as is frequently reported.

Even *D. zeae* has not been important during the last two years. It was not observed in 1950 and in only one field in 1949. Perhaps low temperatures tend to inhibit the pathogenicity of *D. zeae* and *G. zeae*.

In Ontario, stalk rot commonly arises from a rotting of one or more of the roots and this necrosis, after passing through the crown, spreads rapidly up the stalk. All stalk rotting organisms finally produce the same syndrome even the stalk rot of sorghum described by Tullis (16) is very similar. The pith is always destroyed for various distances up the stalks. The stalks assume a dead straw color, are weak and easily compressed, and usually are readily pulled from the ground. Only early examinations and observations will reveal the primary causal organisms. Bacteria are always present during the early stages of necrosis, and *F. moniliforme* is almost always obtained after the plant has been diseased for a considerable time. *P. arrhenomanes* causes much of the corn root rot and frequently spreads up into the stalk.

The importance of *P. pratensis* in the root rot complex is very difficult to assess, but this nematode must cause wounding which enables secondary organisms to enter. In South Carolina, *Pratylenchus zeae* is the species which commonly attacks corn and it, along with *Pratylenchus leiocephalus*, is responsible, according to Graham (7), for brown root rot of tobacco in that State. Probably tobacco in a corn rotation helps to support *P. pratensis*, for Koch and Haslam (10) state: "Brown root rot could, with reasonable certainty, be expected to develop in the tobacco crop, provided one or more satisfactory crops of corn preceded tobacco".

Sometimes wounds made by corn borers (3) serve as portals of entry for corn stalk rotting organisms, but rotting originating in this manner is not nearly so important as that which begins in the roots.

Acknowledgment

The author wishes to thank Dr. L. W. Koch for encouragement throughout this investigation.

References

1. ANDREWS, E. A. and JONES, R. L. Stalk rot of corn in Michigan during 1949. Plant Disease Repts. 34 : 207. 1950.
2. ARK, P. A. Bacterial stalk rot of field corn caused by *Phytomonas lapsa* n. sp. Phytopathology (Abstract), 30 : 1. 1940.
3. CHRISTENSEN, J. J. and SCHNEIDER, C. L. European corn borer (*Pyrausta Nubilalis*) in relation to shank, stalk, and ear rots of corn. Phytopathology, 40 : 284–291. 1950.
4. CHRISTIE, J. R. and PERRY, V. G. A root disease of plants caused by a nematode of the genus *Trichodorus*. Science, 113 : 491–492. 1951.
5. EDWARDS, E. T. Root and basal stalk rot of maize. Agr. Gazette, N.S. Wales, 47: 259–261. 1936.
6. FILIPJEV, J. N. and SHUURMANS-STEKHOVEN, J. H. A manual of agricultural helminthology. E. J. Brill, Ltd., Oude Ryne, Leiden, Netherlands. 1941.
7. GRAHAM, T. W. Nematode root rot of tobacco and other plants. S. Carolina Agr. Expt. Sta., Clemson Agr. Coll. Bull. 390. 1951.
8. JOHNSON, A. G., ROBERT, A. L., and CASH, LILIAN. Further studies on bacterial leaf blight and stalk rot of corn. Phytopathology (Abstract), 35 : 486. 1945.
9. JOHNSON, A. G., ROBERT, A. L., and CASH, LILIAN. Bacterial leaf blight and stalk rot of corn. J. Agr. Research, 78 : 719–732. 1949.
10. KOCH, L. W. and HASLAM, R. J. Variety susceptibility of tobacco to brown root rot in Canada. Sci. Agr. 18 : 561–567. 1938.
11. KOEHLER, B. and BOEWE, G. H. *Gibberella zeae* damage in Illinois in 1946. Plant Disease Reptr. 31 : 169–170. 1947.
12. MCKEEN, W. E. A preliminary study of corn seedling blight in southern Ontario. Can. J. Botany, 29 : 125–137. 1951.
13. ROSEN, H. R. Bacterial stalk rot of corn. Univ. Arkansas, Coll. Agr., Agr. Expt. Sta. Bull. No. 209. 1926.
14. SEMENIUK, G. Development of *Diplodia zeae* and *Gibberella saubinetii* in maize pith following stalk inoculations. Phytopathology (Abstract), 31 : 20. 1941.
15. THORNE, G. On the classification of the *Tylenchida*, new order (Nematoda, Phasmidia). Proc. Helminthol. Soc. Wash. 16 : 37–73. 1949.
16. TULLIS, E. C. *Fusarium moniliforme* the cause of a stalk rot of sorghum in Texas. Phytopathology, 41 : 529–535. 1951.
17. ULLSTRUP, A. J. Corn diseases in Indiana in 1949. Plant Disease Reptr. 34 : 98. 1950.
18. WENHOLZ, H. and DARRAGH, W. H. The root stalk and ear rot diseases of maize—Suggestions for their control. Agr. Gaz. N.S. Wales, 38 : 39–49. 1927.
19. YOUNG, H. C. The tooth pick method of inoculating corn for ear and stalk rots. Phytopathology (Abstract), 33 : 16. 1943.

NOTES

A CONTRAST TECHNIQUE FOR PLAQUE COUNTING*

By G. B. Landerkin[1]

A simple method of increasing the contrast between areas of cell growth and the clear areas of bacteriophage lysis or antibiotic inhibition has been used effectively in this laboratory. It consists of flooding the agar plate with Frazier's reagent (1); a saturated solution of mercuric chloride in 1% hydrochloric acid, allowing contact for one to two minutes, and decanting the excess liquid.

The greater opacity of the treated plates is illustrated in the photograph of plaque-strewn plates of *Pseudomonas pisi*, where the plaques on the right hand (flooded) plate are more sharply outlined. In addition to making the counting of plaques easier the method is effective in preserving the plates and reducing the hazard of bacteriophage dissemination.

1. Frazier, W. C. A new method for the detection of proteolysis by bacteria. J. Bact. 11 : 80. 1926.

Received October 2, 1952.
Contribution No. 348 (Journal Series) from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa.

[1] *Associate Bacteriologist.*

* *Contribution No. 348 (Journal Series) from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa.*

PLATE I

NOTE ON THE TIME POTATO LEAF ROLL VIRUS TAKES TO PASS FROM APHIDS ON THE LEAVES INTO THE DEVELOPING TUBERS*

By R. H. E. Bradley and R. Y. Ganong

Experiments are reported on herein which have been in progress since 1949 to determine the time potato leaf roll virus takes to pass from aphids on the leaves into the developing tubers.

In 1949, 10 rows (35 ft. long) of Foundation A Katahdin tubers were planted in the field at Woodstock, N.B.; in addition, 30 tubers from the same source were cut in halves and the two halves of each planted under a cotton-covered cage designed to keep the plants free from aphids. After the plants commenced to form tubers, from 50 to 100 aphids of the species *Myzus persicae* (Sulz.) that had been reared on young Green Mountain plants infected with leaf roll virus were placed on each plant in one of the outside rows of the plot and under 15 of the cages. Five days after the plants were infested, the plot was sprayed with DDT and the cages were fumigated with nicotine to kill the viruliferous aphids. Eight days after infestation and every four days thereafter, one tuber was taken from each of 20 infested plants in the plot and one from each of 10 infested plants under cages. At the same times, tubers were taken from an equal number of uninfested plants as controls. The tubers were planted in the field the following year and observed for symptoms of secondary leaf roll. The experiments were repeated in 1950 except that tuber samples were also taken at five days after infestation.

In both years, the percentage of tubers infected remained constant in those collected later than 20 days after infestation. Table I shows the numbers of tubers that produced leaf roll plants up to 24 days after infestation. In 1949,

TABLE I

Number of plants which developed leaf roll the season following aphid infestation from tubers left on infested plants for various periods

Days between aphid infestation and tuber sampling	Plants infested 1949, tubers grown 1950				Plants infested 1950, tubers grown 1951			
	Number and percentage of tubers that produced leaf roll plants							
	In open field		In cages		In open field		In cages	
	No.	%	No.	%	No.	%	No.	%
5	—	—	—	—	0	0	0	0
8	10	50	5	50	2	10	7	70
12	16	80	4	40	3	15	7	70
16	20	100	8	80	7	35	10	100
20	20	100	7	70	15	75	10	100
24	20	100	10	100	15	75	10	100

* *Contribution No. 2893, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada.*

half the tubers were infected at eight days; all the tubers collected from plants in the field at 16 days or later were infected, but tubers from the caged plants were not 100% infected until 24 days after infestation. In 1950, 30 tubers taken from plants five days after infestation all produced healthy plants. However, at eight days 70% of the tubers from the caged plants were infected, and tubers from caged plants at 16 days or later were 100% infected. Not all of the plants in the field were infected but, in general, the results were similar to those of 1949. None of the tubers from uninfested plants under cages produced leaf roll plants, and only 5 out of 320 tubers from uninfested plants in the field were infected.

To learn whether all the eyes of newly infected tubers produced leaf roll plants, the tubers produced in the 1950 experiment were cut so that as nearly as possible each seed piece planted contained only one eye. From 64 tubers found to be infected during the first three weeks of sampling, 223 plants were grown and only 6 of these appeared healthy; these six were from four tubers collected at 16 and 20 days after infestation. This result indicates that, even when tubers are removed from the plant soon after becoming infected, nearly all of the eyes produce leaf roll plants.

RECEIVED IN ORIGINAL FORM NOVEMBER 20, 1951,
AND AS REVISED, OCTOBER 10, 1952.
DIVISION OF ENTOMOLOGY,
FIELD CROP INSECT LABORATORY,
FREDERICTON, N.B.

Agricultural Science Abstracts

THE COMMONWEALTH AGRICULTURAL BUREAUX provide a comprehensive abstracting service in the agricultural sciences. A staff of over 100 scientists, translators, and indexers produce some 30,000 abstracts annually, published in a series of journals which are obtainable by subscription. The subjects are:

ANIMAL BREEDING AND GENETICS, ANIMAL HEALTH, ANIMAL NUTRITION, DAIRY SCIENCE, ENTOMOLOGY, FIELD CROPS, FORESTRY, FOREST PRODUCTS AND UTILIZATION, HELMINTH-PARASITOLOGY, HORTICULTURE AND PLANTATION CROPS, MYCOLOGY, PASTURES, PLANT BREEDING AND GENETICS, SOILS AND FERTILIZERS.

From the wealth of material collected Occasional Publications are prepared consisting of reviews of published work, especially on topics of current interest, and descriptions of experimental practice. A catalogue will be sent on application to the Publishing Manager.

COMMONWEALTH AGRICULTURAL BUREAUX
FARNHAM ROYAL BUCKS ENGLAND

CANADIAN JOURNAL OF BOTANY

Notes to Contributors

GENERAL: Manuscripts should be typewritten, double spaced, and the **original and one extra copy** submitted. Style, arrangement, spelling, and abbreviations should conform to the usage of this Journal. Names of all simple compounds, rather than their formulas, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed. Manuscripts should be carefully checked before being submitted, to reduce the need for changes after the type has been set. If authors require changes to be made after the type is set, they will be charged for changes that are considered to be excessive. **All pages, whether text, figures, or tables, should be numbered.**

ABSTRACT: **An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required.**

ILLUSTRATIONS:

(i) *Line Drawings:* All lines should be of sufficient thickness to reproduce well. Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper **ruled in blue only**; any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in **green, yellow, or red should not be used** unless it is desired to have all the co-ordinate lines show. Lettering and numerals should be neatly done in India ink preferably with a stencil (**do not use typewriting**) and be of such size that they will be legible and not less than one millimeter in height when reproduced in a cut three inches wide. All experimental points should be carefully drawn with instruments. Illustrations need not be more than two or three times the size of the desired reproduction, but the ratio of height to width should conform with that of the type page. **The original drawings and one set of small but clear photographic copies are to be submitted.**

(ii) *Photographs:* Prints should be made on glossy paper, with strong contrasts; they should be trimmed to remove all extraneous material so that essential features only are shown. Photographs should be submitted **in duplicate**; if they are to be reproduced in groups, one set should be so arranged and mounted on cardboard with rubber cement; the duplicate set should be unmounted.

(iii) *General:* **The author's name, title of paper, and figure number should be written in the lower left hand corner (outside the illustration proper) of the sheets on which the illustrations appear.** Captions should not be written on the illustrations, but typed together at the end of the manuscript. All figures (including each figure of the plates) should be numbered consecutively from 1 up (arabic numerals). **Each figure should be referred to in the text.** If authors desire to alter a cut, they will be charged for the new cut.

TABLES: Each table should be typed on a separate sheet. Titles should be given for all tables, which should be numbered in Roman numerals. Column heads should be brief and textual matter in tables confined to a minimum. **Each table should be referred to in the text.**

REFERENCES: **These should be listed alphabetically by authors' names, numbered in that order, and placed at the end of the paper.** The form of literature citation should be that used in this Journal. **Titles of papers and inclusive page numbers** of the references cited should be given. **All citations should be checked with the original articles.** Each citation should be referred to in the text by means of the key number.

REPRINTS: A total of 50 reprints of each paper, without covers, are supplied free to the authors. Additional reprints will be supplied according to a prescribed schedule of charges. On request, covers can be supplied at cost.

Approximate charges for reprints may be calculated from the number of printed pages, obtained by multiplying by 0.6 the number of manuscript pages (double-spaced typewritten sheets, 8½ in. × 11 in.) and making allowance for space occupied by line drawings and halftones (not inserts). The cost per page is tabulated at the back of the reprint request form sent with the galley.

Contents

Page

The Growth Cycle of the Blueberry and Some Factors of the Environment —*Hugh P. Bell* - - - - - - - - - - - - 1

An Essential Bacterial Growth Factor Produced by Microbial Synthesis —*A. G. Lochhead and Margaret O. Burton* - - - - - - - 7

The Use of a Percolation Technique in Studying Antibiotic Production in Soil—*I. L. Stevenson and A. G. Lochhead* - - - - - - - 23

Production of Cytases Active on Barley Gum by Bacteria of the Genus *Bacillus* —*A. C. Blackwood* - - - - - - - - - - - - 28

The Response of Potato Tubers to a Period of Anaerobiosis. I. Drifts in Carbon Dioxide Production—*Dorothy F. Forward* - - - - - 33

Further Observations on the Reduction of Fusiform Cambial Cells in *Thuja occidentalis* L.—*M. W. Bannan* - - - - - - - - - 63

The Isolation and Identification of Sucrose from the Seedling Wheat Leaf —*G. M. Ward* - - - - - - - - - - - - 75

Physiological and Biochemical Studies in Plant Metabolism. VII. The Invertase of the Seedling Wheat Leaf—*G. M. Ward* - - - - - 81

Microbiological Studies of Air over some Nonarctic Regions of Canada —*C. D. Kelly and S. M. Pady* - - - - - - - - - - 90

Studies on Microorganisms in Arctic Air During 1949 and 1950—*S. M. Pady and C. D. Kelly* - - - - - - - - - - - - 107

A Further Survey of Plants for Antibacterial Substances—*R. E. MacDonald and C. J. Bishop* - - - - - - - - - - - - 123

Preliminary Studies of Root and Basal Stalk Rot of Maturing Corn in Ontario *W. E. McKeen* - - - - - - - - - - - - 132

A Contrast Technique for Plaque Counting—*G. B. Landerkin* - - - - 142

Note on the Time Potato Leaf Roll Virus Takes to Pass from Aphids on the Leaves into the Developing Tubers—*R. H. E. Bradley and R. Y. Ganong* - 143